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RESEARCH PAPER

Effects of whey protein and azadirachtin on *Bemisia tabaci* (Hemiptera: Aleyrodidae) and *Tomato yellow leaf curl virus*

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ABSTRACT

A research work was conducted to develop alternative method instead of using synthetic insecticides to control whitefly (*Bemisia tabaci*) and TYLCV under the field condition. Azadirachtin (Extract of neem leaves) and whey protein (Esterification from cow milk) are non-toxic and relatively harmless to human. Therefore, four treatments, control (T0), azadirachtin with whey protein together (T1), azadirachtin only (T2), and whey protein only (T3) with four replications of each treatment following RCBD design were sprayed of solution @1ml/L at 7 days interval after initial releasing of 100 TYLCV-viruliferous whiteflies in each replication. The number of whiteflies were significantly decreased in T1 (62.50±6.81) and significantly increased in T0 (230.50±6.81). The maximum height of plants (97.50±2.68 cm) were found in T1 and the minimum height (82.50±2.68 cm) of same aged plant were found in T0.The highest number of leaves (57.75±1.37) was recorded in T1 and the lowest number of leaves (25.00±1.37) in T0. Moreover, the maximum number of branch at 30, 60 and 90 DAS were found 7.25, 11.25 and 14.00 in T1 and 4.75, 6.00 and 6.00 in T0 respectively compared to others. The highest number of tomato (39.25±1.20) and average weight of per tomato (60.63±1.20 gm) were recorded in T1 which followed by the number of fruits (35.25±1.20) and average weight of per tomato (58.72±1.20 gm) in T2 compare to others. As a result, combined effects of azadirachtin and whey protein shown better performance to suppress the population of whiteflies and the growth of TYLCV.

Key words: Control, neem, TYLCV, whey protein, whitefly

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is an important member of the Solanaceae family. It is a nutritious, delicious and highly popular vegetable crop in Bangladesh due to its high nutritive value, taste and versatile use in various food items around the World and also a vital source of higher contents of vitamin A, B and C including calcium, iron and other minerals (Opena et al., 1987; Bose & Som, 1990). Lycopene in tomato is influential antioxidant that can reduces the risk of prostate cancer (Hossain et al., 2004). In Bangladesh, it is widely grown in winter and to some extent in summer season. About 23827.53 hectares of land were occupied under tomato cultivation, producing 190213 Metric ton fresh fruits in the year 2009-2010 (BBS, 2010). Although the total cultivated area and production of tomato in our country have increased gradually over the last few years but the productivity is still very low (7.98 m ton hectare^{-1}) compared to the average of the world yield (26.29 t ha^{-1}) as per FAO (2003). Globally tomato is susceptible to more than 200 diseases, out of which 40 are caused by viruses (Martelli & Quacquarelli, 1982; Lukyanenko, 1991). To this point, 16 different viruses have been recorded on tomato in Bangladesh (Akanda et al., 1991; Akanda, 1994). Among these viral diseases, *Tomato Yellow Leaf Curl Virus* (TYLCV) is considered as the most devastating one (Kalloo, 1991) and whitefly (*Bemisia tabaci*) known as single vector for spreading of TYLCV. In order to control TYLCV disease, it was found that repeated spray at 7 days interval of insecticide, like Cypermethrin (0.01%) or Dimethoate (0.1%) is effective to eradicate the disease by controlling its vector (Fadl & Burgstaller, 1984; Fanigliulo et al., 2009).

TYLCV causes severe damage on tomatoes. This disease is ssDNA plant virus, which belongs to the family Geminiviridae of the genus Begomovirus. The virus is mechanically non- transmitted, but generally transmitted in the fields on infected transplants by grafting and then it spread plant to plant by whitefly,

which could be able to infect plants in any stage of plant growth in a semi constant approach (Green & Kalloo, 1994; Ghanim et al., 1998; Gupta, 2000). TYLCV threatens both commercial tomato production in the fields and home garden. The virus can cause up to 100% yield loss in tropics and subtropics depending upon severity and stage of infection (Green and Kalloo, 1994). The characteristic symptoms of TYLCV infected plant includes chlorotic margin, upward leaf cupping, leaf mottling, reduced leaf size, stunted growth and flower drop (Gafni, 2003; Green & Kalloo, 1994).

B. tabaci is a very complex species consists of at least 24 biotypes in tropical and sub-tropical region around the World (Boykin et al., 2007). There are three genotypic cluster of indigenous whitefly BW1, BW2 and BW3 were identified from Bangladesh (Jahan et al., 2015). This devastating global insect pest caused damage directly by sucking the plant sap from phloem, indirectly by excreting honeydews that produce sooty mould, and by spreading 111 plant virus diseases (Jones, 2003). Very recent Jahan et al. (2014) reported that TYLCV inoculated whitefly was caused more damage to tomato plants by feeding and frequent probing.

From the very beginning, several scientists recommended the use of milk as spraying or dipping of seedlings for reducing the incidence of virus infections. Recent studies demonstrated the effectiveness of milk in reducing infection of tobacco mosaic virus (TMV) in pepper, tomato, and tobacco (Gillian, 2005). Various components with fascinating efforts for anti-TLCV characteristics of milk and its derivatives (whey protein) have recently come forward as successful approach for reducing TLCV infection (Chobert et al., 2007). Whey represents a rich and heterogeneous mixture of secreted proteins with wide ranging nutritional, biological and food functional attributes. The main constituents of whey are α-lactalbumin (ALA), β-lactoglobulin (BLG) and two small globular proteins that account for approximately 70-80% of total whey protein (Jovanović et al., 2005). Esterified whey proteins fractions have been effective in inhibiting TYLCV infection in tomato plants. Possibly, these proteins may saturate the binding of viral DNA to inhibit viral replication process causing TYLCV inhibition (Abdelbacki et al., 2010).

Neem is active at low concentrations, has negligible mammalian toxicity, degrades rapidly in the environment and it proved in most cases not to be harmful, or only slightly harmful, to important natural enemies of pests (Schmutterer, 1990). Combined application of Neem extract and cow dung was more effective to increase the growth of okra plant and yield production of okra (Sale et al., 2015). The modified neem leaf extract also increased the plant height and stem girth of maize, and was shown the most effective in improving soil fertility, growth and yield of maize and watermelon (Moyin-Jesu, 2012).

Current research is focused on the use of alternative method to avoid the undesirable effects of the insecticides. Nowadays farmers were faced severe infection with TYLCV in tomato fields in Bangladesh. But they don't know proper mechanism for virus management due to lack of appropriate knowledge. Keeping in mind the above mentioned problem, the current research work has been planned to understand the effective whitefly management and eradicate the TYLCV infection friendly to environment by low of cost in Bangladesh that will be helpful for the farmers of our country to take proper management strategies. Conversely, farmers gain the economic sustainability by the quality production**.**

Materials and methods

The present study regarding effects of whey protein and azadirachtin on whitefly (*B. tabaci)* and Tomato yellow leaf curl virus in Tomato cultivar has been conducted during October 2015 to March 2016 at the experimental fields of Patuakhali Science and Technology University, Patuakhali. There were four treatments, spraying of water only as control (T0), spraying of both Azadirachtin and whey protein together @1ml/L (T1), spraying of individual Azadirachtin @1ml/L (T2), and spraying of individual whey protein @1ml/L (T3) at seven (7) days interval with four replications of each treatment following RCBD design were applied after initial releasing of 100 TYLCV-viruliferous whiteflies in each replication. Required materials and methodology are described below under the following heading.

Land Preparation

The soil was well prepared and good tilth was ensured for commercial crop production. The land of the experimental field was ploughed with a power tiller. Later on the land was ploughed three times followed by laddering to obtain desirable tilth. The corners of the land were spaded and larger clods were broken into smaller pieces. After ploughing and laddering, all the stubbles and uprooted weeds were removed and then the land was ready. The field layout and design of the experiment were followed immediately after land preparation. The target land was divided into 4 equal plots (1m×1.5m) with plot to plot distance of 1.0 m and block to block distance is 1.0 m.

Collection of seed, seedling raising and transplanting

The tomato seeds of "Raton" variety were collect from Bangladesh Agricultural Research Institute (BARI). Seeds were then directly sown in the middle of October, 2015 in seedbed containing a mixture of equal proportion well decomposed cowdung and loam soil. Seeds were sown in seedbed and irrigated regularly. After germination the seedling were sprayed with water by a hand sprayer. Watering was done 3 or 4 times in a week. Seedlings were placed in a shady place for transplanting in the main field. Thirty days old healthy seedlings were transplanted in the pits of the main field. Other intercultural operations were done as mentioned earlier.

Azadirachtin extraction

Fresh neem leaf was collected from the PSTU campus from the local neem tree. The fresh neem leaves were cleaned into the tap water. A weigheted quantity of dried neem leaves were blended until it turns into the paste form. Then the paste form of neem leaves were put into a beaker and stored in 4^0C temperature in the

refrigerator. Then it was collected into a test tube and centrifuged at 10 minutes at 10000 rpm. After 10 minutes the test tube were removed from the centrifuge machine and collect the liquid from the tube and discarded the solid portion from the tube. Then again the liquid portion took into another test tube and centrifuge it again for 10 minutes at 10000 rpm. Then again the liquid portion was taken and the solid portion was discarded. And this liquid was used as azadirachtin.

Whey protein esterification

500ml of cow milk were taken in a beaker. Then added 2.5 ml of HCL into the milk and stirring it for a while. HCL must be added drop by drop and stirred the milk. Kept it into 4^0C temperature in the refrigerator at 12 hours. Then it took it into the test tube and centrifuged at 10 minutes 10000 rpm. Then the resulting supernatant was discarded and the residue was dispersed in a volume of alcohol (99.7%) equal to that of the discarded supernatant and well mixed before re-centrifuging at the same conditions. This washing steps was repeated three times. The final precipitate was dissolved in an appropriate amount of distilled water then submitted to freeze drying. The lyophilized samples were kept at -20℃ until analysis.

Cultural practices

After transplanting, a light irrigation was given. Subsequent irrigation was applied in all the plots as and when needed. After 15 days of transplanting a single healthy seedling and luxuriant growth per pit was allowed to grow discarding the others, propping of each plant by bamboo stick was provided on about 1m height from ground level for additional support and to allow normal creeping. Weeding and mulching in the plot were done, whenever necessary.

Efficacy of Treatments for the Virus Transmitting Whitefly

The sampling on the incidence of whitefly and the occurrence of TYLCV was done by direct visual method (Hirano et al., 1993). The sampling of the incidence of whitefly was taken at vegetative, early flowering, early fruiting and fruit ripening stages at 15 days interval. The plants were carefully checked visually for the presence of whitefly. Sometimes plants were shaken gently to observe their presence and count their number accurately. As the population of whitefly was very low the number was recorded per 4 plants. Sampling on whitefly incidence was taken at both pre and post application of treatments. Two post treatment counts were taken at each vegetative, early flowering, early fruiting and at fruit ripening stages. The effectiveness of each treatment in reducing the infestation of whitefly and suppressing the infection of virus diseases was evaluated on the basis of some preselected parameters such as, number of leaves and it's length, number of whiteflies, plant height, number of fruits and it's weight, number of healthy and deformed fruits, number of branches.

Rearing of whitefly in the laboratory:

Samples of non-viruliferous adult whiteflies were collected from Patuakhali on tomato host plant in Bangladesh and some of them were immediately preserved in 99% ethanol. Rest of collected alive samples were brought to PSTU lab (Entomology laboratory in Patuakhali Science and Technology University) with a host plant in a rearing chamber for making vector colonies one for non-viruliferous and another for viruliferous using square metallic structure with insect proofed fine mesh. These two colonies were reared in two separate insect rearing chambers under condition of 25± 2℃ and 60±5% relative humidity and 16 h light/8 h dark photoperiodic cycle as described by Park et al., 2012.

Data Collection and Calculation

For data collection 4 plants per treatment were randomly selected and tagged. Data collection was started at 15 days after spraying (15 DAS) the seedlings and continued up to fruit set. All the data were collected once in a week. The data were collected on number of whitefly per plant; tomato yellow leaf curl infected plant per plot, number of branches per plant, fruit bearing capacity per plot, total weight of healthy fruit per plant (kg), total weight of deformed fruit per plant (kg), total number of healthy and deformed fruit per plant, percent fruit deformation, total weight of fruit per plant (kg).

Statistical analysis

The data obtained for different characters were statistically analyzed to find out the incidence of whitefly, diseases severity and effect on the yield of tomato. The mean values of all the characters were calculated and analysis of variance was performed by using the "F" (variance ratio) test. The significance of the difference among the treatment combinations means ware determined by the Duncan's Multiple Range Test (DMRT) at 5% level of probability.

Results and discussion

An experiment was performed to investigate the effect of whey protein and azadirachtin on whiteflies and TYLCV. The analysis of variance (ANOVA) of the data on fruit infestation and different yield contributing characters are given in the result has been presented and discussed and possible interpretations have been given under the following headings.

Statistically significant variation was recorded in number of leaves per tomato plants by the combined effect of whey protein and azadirachtin on the tomato plants growth under different treatments. Highest number of leaves per plant (57.75±1.37) was recorded (Table 1) in T1 consisting of whey protein and azadirachtin (1ml + 1ml)/ litre of water at 7 days interval which was closely followed (43.25±1.37) was recorded (Table 1) in the treatments of T2 consisting of azadirachtin1ml/ litre of water at seven days interval. On the other hand the lowest number (25.00±1.37) of leaves per plant was recorded (Table 1) in the T0. The result showed that the highest number of leaves were recovered in the under treatment of combined effects of

Table 1. Number of leaves, maximum and minimum length of leaves per tomato plant as effected by various treatments

Treatments	Number of leaves (n)	Length of leaves (cm)	
		Maximum	Minimum
T0 (Control)	25.00±1.37 d	13.50±0.48 c	6.50±0.37 a
T1 (Whey protein and azadirachtin)	57.75±1.37 a	21.25±0.48 a	7.75±0.37 a
T2 (Azadirachtin)	43.25±1.37 b	16.00±0.48 b	7.25±0.37 a
T3 (Whey protein)	32.00±1.37 c	15.75±0.48 b	6.50±0.37 a
Significance	***	***	NS
CV	6.52	5.76	10.65
SE(±)	1.3693	0.4787	0.3727

Means followed by same letter in a column are not significantly different at 5 % level by DMRT
SE (±) = Standard error of means
CV= Coefficient of variation
***= Significant at 0.1% level

whey protein and azadirachtin. Same trend of result in the number of leaves had been found by Avgelis et al. (2001).

The maximum length of leaves was recorded in per tomato plant by the effect of whey protein and azadirachtin on the growth of tomato plants and TYLCV under the various treatments. The largest leaves (21.25±0.48 cm) of tomato plants were counted (Table 1) in the treatment T1 of whey protein and azadirachtin in consisting of (1ml+1ml)/ litre of water at seven days interval. And the second largest leaves (16.00±0.48) are counted (Table 1) in the treatment T2 which is consists of azadirachtin in amount of 1ml/litre of water at seven days interval. The minimum length of leaves was recorded in per tomato plant to determine the effect of whey protein and azadirachtin on the growth of plants and TYLCV under the various treatments. The smallest leaves (6.50±0.37 cm) of tomato plants are counted (Table 1) in the treatment T0 which treated as control and the T3 which was consists of whey protein of 1ml/ litre of water at seven days interval. Sale et al. (2015) reported about effects of neem extract on the growth of okra that matched with current study. Severe TYLCV infection were found in T0 but TYLCV free found in T1 that shown similar effects by Chobert et al. (2007) reported that various components with fascinating efforts for anti-TLCV characteristics of milk and its derivatives (whey protein) have recently come forward as successful approach for reducing TLCV infection.

Statistically significant variation was recorded in height of tomato plants under different treatments. The maximum height of plant (97.50±2.68 cm) was recorded (Table 2) in T1 consisting of whey protein and azadirachtin (1ml + 1ml)/ litter and the second tallest plant (87.00±2.68 cm) was recorded (Table 2) in T2 consisting of azadirachtin 1ml/ litre which was closely followed (85.50±2.68 cm) was recorded (Table 2) in T3 consisting of whey protein 1ml/ litre. On the other hand the minimum height (82.50±2.68 cm) of leaves per plant was recorded (Table 2) in T0 treatment. The result showed that the tallest plants were counted in the treatment of both whey protein and azadirachtin together (T1). And also whey protein had the similar effect as like as azadirachtin. In the effect of azadirachtin was shown the height of tomato plant (87.00±2.68 cm) in T2 treatment consisting of 1ml/ litre at seven days interval. Same kind of result in the plant height had been found by Pruthi and Samuel (1942). The result supported with Sale et al. (2015) and Moyin-Jesu (2012) by their findings about Neem extract on effective to increase the growth of plants.

Table 2. Height of tomato plants as affected by various treatments

Treatment	Plant height (cm)
T0 (Control)	82.50±2.68 b
T1 (Whey + Azadirachtin)	97.50±2.68 a
T2 (Azadirachtin)	87.00±2.68 b
T3 (Whey protein)	85.50±2.68 b
Significance	**
CV	6.08
SE(±)	2.6818

Means followed by same letter in a column are not significantly different at 5 % level by DMRT
SE (±) = Standard error of means
CV= Coefficient of variation
**= Significant at 0.01% level

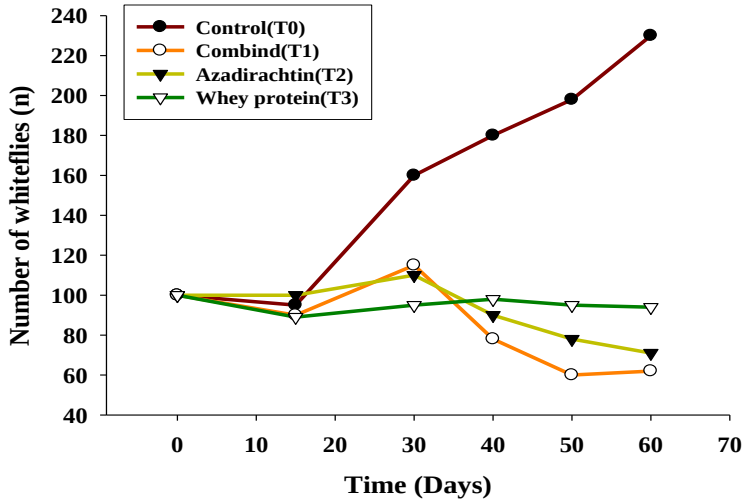

Figure 1. Effect of different spray on the number of whiteflies in different treatments

Statistically significant variation was recorded in number of whiteflies per tomato plants to determine the effect of whey protein and azadirachtin on the population of whiteflies (*B. tabaci*) under different treatments. Highest number of insects 95, 160, 180, 195 and 230 were recorded (Figure 1) in T0 by the counting of 15, 30, 40, 50 and 60 days after released respectively. On the other hand the lowest number of insects 90, 115, 78, 60 and 62 were recorded (Figure 1) in the T1 by the counting of 15, 30, 40, 50, 60 days respectively. The result showed that the large amount of insects were counted in the Control treatment (T0). These results indicate the efficacy of neem extract which can significantly decreased the number of whitefly population, same findings mentioned by Medina et al. (2004) for its antifeedant action (Blaney et al., 1990), with negative effects on reproduction have also been reported in a number of insects (Schmutterer & Rembold 1995) and reduce of vitellogenin synthesis in insects which caused fecundity decrease (Medina et al., 2004) that fully matched with current study.

Table 3. Effect of different treatment on number of fruits, maximum and minimum fruit weight per plant

Treatments	Number of fruits/ plant (n)	Weight/ Fruit (gm)	Fruit weight (Kg) / plant	
			Maximum	Minimum
T0 (Control)	21.75±1.20 d	42.21±1.20 c	1.05±0.05 b	0.43±0.04 c
T1 (Whey + Azadirachtin)	39.25±1.20 a	60.63±1.20 a	2.38±0.05 a	1.32±0.04 a
T2 (Azadirachtin)	35.25±1.20 b	58.72±1.20 a	2.07±0.05 a	1.27±0.04 a
T3 (Whey protein)	30.50±1.20 c	51.13±1.20 b	1.59±0.05 b	0.82±0.04 b
Significance	***	***	***	***
CV	5.42	5.42	7.62	6.74
SE(±)	1.1968	1.1968	0.0486	0.0356

Means followed by same letter in a column are not significantly different at 5 % level by DMRT
SE (±) = Standard error of means
CV= Coefficient of variation
***= Significant at 0.1% level

The number of fruits was recorded in per tomato plant to determine the effect of whey protein and azadirachtin on yield production under the various treatments. The highest number (39.25±1.20) of tomato fruits and average weight of per tomato (60.63±1.20) were recorded (Table 3) in T1. And the near about the same number of fruits (35.25±1.20) and average weight of per tomato (58.72±1.20) were found (Table 3) in T2. The lowest numbers of fruits (21.75±1.20) and average weight of per tomato (42.21±1.20) were counted (Table 3) in the treatment of T0. So the result showed that the number of fruits and weight of fruits were calculated highest (Table 3) in the T1and nearly so in T2 treatment.

The largest fruit was recorded in per tomato plant by the effect of whey protein and azadirachtin. The maximum fruit weight (2.38±0.05, 2.07±0.05, 1.59±0.05 and 1.05±0.05) and the minimum fruit weight (1.32±0.04, 1.27±0.04, 0.82±0.04 and 0.43±0.04)Kg per tomato plantwerecounted in the treatment T1, T2, T3 and T0 respectively(Table 3).So the result were shownthe yield productivity increased in the T1which followed by T2 treatment. So it was clear that combined

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effect (T1 Treatment) get the result better than T0and T3 treatment. Similar findings were mentioned by Sale et al. (2015) and Moyin-Jesu(2012) usingneem extractcan easily improving soil fertility, growth of plants and yieldproduction in field. Gillian (2005) demonstrated the effectiveness of milk in reducing infection of tobacco mosaic virus in pepper, tomato, and tobacco that also matched with the current findings which followed by Abdelbacki et al. (2010).

The number of branches were recorded in per tomato plant at 30 DAS to determine the combined effect of whey protein and azadirachtin on TYLCV growth under the various treatments. The total numbers of brunches of tomato plant at 30 DAS were counted (Table 4) in the treatment T0, T1, T2 and T3 were (4.75±0.26), (9.25±0.26), (5.50±0.26) and (6.25±0.26) respectively. The total number of branches of tomato plant at 60 DAS were counted (Table 4) in the treatment T0, T1, T2 and T3 were (8.00±0.42), (14.25±0.42), (9.50±0.42) and (11.00±0.42) respectively. The total number of branches of tomato plant at 90 DAS were counted (Table 4) in the treatment T0, T1, T2 and T3 were (11.00±0.29), (21.00±0.29), (11.75±0.29) and (14.00±0.29) respectively. Same findings were also mentioned by Sale et al. (2015) that neem extract effectively increased the plant growth. Moyin-Jesu(2012) reported also aboutneem leaf extract whichincreased the plant height and stem girth of maize, and was shown the most effective in improving soil fertility, growth and yield of maize and watermelon.

Table 4. Effect of different treatment of number of branch at 30 DAS, 60 DAS and 90 DAS

Treatments	Number of branches (Days)		
	30	60	90
T0 (Control)	4.75±0.26 c	6.00±0.42 c	6.00±0.29 c
T1 (Whey protein and azadirachtin)	7.25±0.26 a	11.25±0.42 a	14.00±0.29 a
T2 (Azadirachtin)	5.50±0.26 b	8.50±0.42 b	11.75±0.29 b
T3 (Whey protein)	6.25±0.26 a	10.00±0.42 a	12.50±0.29 b
Significance	***	***	***
CV	8.29	7.99	4.04
SE(±)	0.2668	0.4270	0.2917

Means followed by same letter in a column are not significantly different at 5 % level by DMRT
SE (±) = Standard error of means
CV= Coefficient of variation
***= Significant at 0.1% level.

Table 5. Effect of different treatments on % of healthy and deformed fruits

Treatment	Healthy fruit (%)	Deformed fruit (%)
T0 (Control)	39.28±2.28 d	60.72±0.76 a
T1 (Whey +Azadirachtin)	91.35±2.28 a	8.727±0.76 d
T2 (Azadirachtin)	77.05±2.28 b	21.45±0.76 c
T3 (Whey protein)	67.05±2.28 c	32.70±0.76 b
Significance	***	***
CV	6.42	5.42
SE(±)	2.2861	0.7691

Means followed by same letter in a column are not significantly different at 5 % level by DMRT
SE (±) = Standard error of means
CV= Coefficient of variation
***= Significant at .01% level

Statistically significant variation was recorded of healthy and deformed tomato production by the effect of whey protein and azadirachtin under different treatments. The production of healthy and deformed fruits (%) in tomato plants were counted (Table 5) in the treatment T0,T1, T2 and T3 and found 39.28±2.28, 91.35±2.28, 77.05±2.28, 67.05±2.28and 60.72±0.76, 8.727±0.76, 21.45±0.76 32.70±0.76 respectively. Moyin-Jesu(2012) reported same as the current findings that neem extract was shown more effective to increase the yield production of maize and watermelon which followed same findings in different crops by Sale et al. (2015) on okra.Recent studies demonstrated the effectiveness of whey proteinon inhibition the growth of TYLCV in tomato (Abdelbacki et al. 2010) that supported the current findings.

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References

Abdelbacki AM, Taha SH, Sitohy MZ, Dawood AIA, Abd-El Hamid MM, Rezk AA (2010) Inhibition of Tomato Yellow Leaf Curl Virus (TYLCV) using whey proteins. *Virol. J.*, 7:26.

Akanda AM, Tsuno K, Wakimoto S (1991) Serological detection of four plant viruses in cucurbitaceous crops from Bangladesh. *Ann. Phytopath. Sco. Japan*., 57: 499–505.

Akanda AM (1994) Virus diseases of tomato and eggplant and their control. Lecture note for the trainers training programmes on grafting technology of tomato and eggplant. IPSA–JICA publication. IPSA.Salna, Gazipur. pp. 28–33.

Akanda AM, Alam N, Khair A (1999) Identification and distribution of Virus diseases of tomato in Bangladesh. *Bangladesh J. Life Sci*., 11: 42-48.

Avgelis AD, Roditakis N, Dovas CI, Katis NN, Varveri C, Vassilakos N, Ben, F (2001) First report of *Tomato yellow leaf curl virus* on Tomato crops in Greece. *Plant Disease*, 85: 678.

BBS (Bangladesh Bureau of Statistics), 2010.Hand book of agricultural statistics. Estimate of Tomato 2009-2010. Bangladesh Bureau of Statistics, Ministry of Planning, Govt. Peoples Repub. Bangladesh, pp: 4/14.

Blaney WM, Simmonds MJJ, Ley SV, Anderson JC, Toogood PL (1900) Antifeedant effects of aza and structurally related compounds on lepidopterous larvae. *Entomol. Exp. Appl.*, 55:149–160.

Bose TK, Som MG (1990) Vegetable Crops in India. B. Mitra and NayaProkash, 206 BidhanSarani, Calcutta, India, pp: 249.

Boykin LM, Shatters RG, Rosell RC, McKenzie CL, Bagnall RA, De Barro, PJ, Frohlich DR (2007) Global relationships of *Bemisiatabaci* (Hemiptera: Aleyrodidae) revealedusing Bayesian analysis of mitochondrial COI DNA sequences. *Mol. Phylogenet. Evol.*, 3:1306–1319.

Chobert JM, Sitohy M, Billaudel S, Dalgalarrondo M, Haertlé T (2007) Anticytomegaloviral activity of esterified milk proteins and L-polylysines,.*J Mol. Microbiol.Biotechnol.*,13: 255-258.

Fadl GM, Burgstaller H (1984) Reduction of tomato leaf curl virus in Sudan through variety selection and insecticide application. *Acta Horticulture*, 190:159-164.

Fanigliulo A, Comes S, Crescenzi A, Momol MT, Olson SM, Sacchetti M, Ferrara L, Caligiuri G (2009) Integrated management of tomato yellow leaf curl in protected tomato crops in southern Italy. *Acta Horticulture* (ISHS), 808:393-396.

FAO (2003) FAO, Production Yearbook. Vol. 57. p. 140–141

Gafni Y (2003) *Tomato yellow leaf curl virus*, the intracellular dynamics of a plant DNA virus. *Mol. Plant pathol.* 4 (1): 9-15.

Ghanim M, Morin S, Zeidan M, Czosnek H (1998) Evidencefor transovarial transmission of tomato yellow leaf curl virusby its vector, the whitefly *Bemisia tabaci*. *Virology*, 240:295-303.

Gillian F (2005) Milk as a Management Tool for Virus Diseases.http://www.omafra.gov.on.ca/english/crops/hort/news/grower/2005/11gn05a1.htm.

Green SK, Kalloo G (1994) Leaf curl and yellowing diseases of tomato. In: Technical Bulletin No. 21. Leaf curl and yellowing viruses of pepper and tomato. In an overview Asian Vegetable Research and Development Centre 21-51 pp.

Gupta ND (2000) Occurrence of *Tomato yellow leaf curl virus* (TYLCV) and *Tomato purple vein virus* (TPVV) and their effect on growth and yield of tomato. As an MS thesis submitted to the Department of Plant Pathology, BSMRAU, Salna, Gazipur, Bangladesh. 77p.

Hirano K, Budiynto E, Winarni S (1993) Biological characteristics and forecasting outbreaks of the whitefly *Bemisiatabaci*, a vector of virus diseases in soybean fields.Food and Fertilizer Technical bulletin.No.135.

Hossain MM, Khalequzzaman KM, Hossain MA, Mollah MRA, Siddique MA (2004) Influence of planting time on the extension of picking period of four tomato varieties. *J. Biological Sci.*, 4: 616-619.

Jahan SMH, Lee K-S, Lee S, Lee K-Y (2014) Upregulation of probing and feeding related behavioral frequencies in *Bemisiatabaci* upon acquisition of *Tomato yellow leaf curl virus*. *Pest Management Science*, 70(10): 1497-502.

Jahan SMH, Lee K-Y, Howlader, MIA (2015) The third genotypic clusters of *Bemisiatabaci*(Gennadius) (Hemiptera: Aleyrodidae) found in Bangladesh. *Bangladesh J. Agril. Res.*, 40(1): 1-16.

Jones DR (2003) Plant viruses transmitted by whiteflies. *Eur. J. Plant Pathol.*,109 (3): 195–219.

Jovanović S, Miroljub B, Ognjen M (2005) Whey proteins-Properties and Possibility of Application. *Mljekarstvo*, 55:215-233.

Kalloo G (1991) *Genetic improvement of tomato*. Springer verlag, Berlin Heidelberg, Germany. p. 358.

Lukyanenko AN (1991) *Disease Resistance in Tomato*. In: Kalloo G (ed.) 1991. Genetic improvement of tomato. Springer verlag, Berlin Heidelberg, Germany. pp. 99–119

Martelli GP, Quaequarelli A (1982) The present status of tomato and pepper viruses. *Acta Horticulturae*, 127: 39-64.

Medina P, Budia F, Del Estal P, Viñuela E (2004) Influence of Azadirachtin, a Botanical Insecticide, on *Chrysoperlacarnea* (Stephens) Reproduction: Toxicity and Ultrastructural Approach. *Journal of Economic Entomology*, 97(1): 43-50.

Moyin-Jesu EI (2012) Comparative evaluation of modified neem leaf, neem leaf and woodash extracts on soil fertility improvement, growth and yields of maize (*Zea mays* L.) and watermelon (*Citrulluslanatus*) (sole and intercrop). *Agricultural Sciences*, 3: 90-97

Opena RT, Luo CG, Yoon JY (1987) Breeding for stress tolerance under tropical conditions in tomato and heading Chinese cabbage. ASPAC-FFTC. Taipei, Taiwan (ROC). Pp.88-109.

Park J, Jahan SMH, Song W-G, Lee H, Lee Y-S, Choi H-S, Lee K-S, Kim C-S, Lee S, Lee K-Y (2012) Identification of biotypes and secondary endosymbionts of *Bemisia tabaci* in Korea and relationships with the occurrence of TYLCV disease. *Journal of Asia–Pacific Entomology*, 15: 186–191.

Pruthi HS, Samuel CK (1942). Entomological investigation on the leaf curl disease of tobacco in Northern India. V. Biology and population of the whitefly vector (*Bemisiatabaci*Genn.) in relation to the incidence of the disease. *Indian J. Agric. Sci*,. 12:35-57.

Sale FA, Fadipe TF, Adah H (2015) Effect of neem extract preparations and nutrient sources on performance of okra (*Abelmoschusesculentus*) plant. *Nigerian Journal of Agriculture, Food and Environment,* 11(2):221-225.

Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree *Azadirachtaindica*. *Annu. Rev. Entomol.*, 35:271–297.

Schmutterer H, Rembold H (1995) Reproduction. H. Schmutterer The neem tree. VCH Weinheim, Germany.