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Sowing Date Effect on Tomato Yellow Leaf Curl Virus

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ABSTRACT: Tomato Yellow Leaf Curl Virus (TYLCV) is one of usual ailments which changing sowing date, as one of appropriate agricultural methods, has significant effect on damage reduction of mentioned disease and chemical fertilizer application. According to no report and research on the best sowing date of tomato in south of Kerman, this research was conducted for nomination the best sowing date in order to escape TYLCV and reduce its effect in open air cultivation in Manoujan of Kerman. It was done in the form of Complete Block Design with five treatments and three replications. Seeds were cultivated in terrarium (6 August, 21 August, 5 September, 20 September and 5 October) and based on the plant phenology (5-6 leaf stage) transplanted in the main land. The results indicated that sowing in early August to September could not reduce the damage severity but planting in 20 September and 5 October significantly reduced pollution percent and severity.

Keywords: TYLCV, transplanting, sowing date, tomato, viral disease

INTRODUCTION

Tomato, as one of the important agricultural crops in South of Kerman Province, is cultivated under open air condition/short plastic tunnels. Numerous pests and fungal, bacterial and viral disease are some of its production and cultivation problems in the mentioned area of Iran. Tomato Yellow Leaf Curl Virus (TYLCV) is one of usual ailments of the crop in Kerman, Iran. According to reports of some researchers, TYLCV is one of the most dangerous viral pathogens in tropical and sub-tropical rejoins of Africa, Asia, Mediterranean and Middle East (Lapidot and Polston, 2006). For the first time, outward signs of TYLCV were reported in occupied Palestine in 1964 but its inducing factor was not recognized until 1988 (Lapidot and Polston, 2006). The disease spread in many tropical and sub-tropical countries of the world such as Palestine, Ivory Coast, Syria, Lebanon, Senegal, Egypt, Tunisia, India, Cuba, Mexico, Guatemala, Honduras, Nicaragua, Venezuela, Costa Rica, Brazil and Iran, and import substantially damage to tomato fields each year (Blancard, 1992). According to the tomato cultivars sensitivity, populations of insect vector and climatic condition, damage percent and yield reduction had been reported between 20 and 100 percent (Zitter, 1991; Capinera, 2001). Due to widespread pollution of tomato fields of Jordan, tomato cultivation has declined from 14.6 to 5.6 hectares. This disease mostly damages tomato fields in summer and autumn, and lead to complete loss of product in occupied Palestine; In addition, all fields of autumn cultivation are under attack of the viral disease and it could damage products amounting to 80% in Egypt (Bananj and Ahoun Manesh1999). The outward symptoms, host ranges and possibility of transmission with the sap in infected plants is distinguishable and indivisible from other viral disease such as TGMV, TYMV and TMV which transfer by white flies (Zitter, 19991). This virus is a Gemini virus (twin virus) that transfers by *Bemisia tabaci* in the wild.

This virus was reported for the first time in Hormozgan and Sistan-Baluchestan Provinces of Iran and identified as TYLCV in 1994 (Hajimorad *et al.*, 1994). Subsequently, it was reported from Tehran and Khorasan Provinces in 1998 (Bananj and Ahoun Manesh1999). The disease is already observed in many of tomato fields, cultivation under plastic/greenhouse of southern part of Iran (Bananj and Ahoun Manesh1999; Azadvar and Seyfi, 2006). Disease scathe has been reported between 70 to 90% in some tomato fields of Hormozgan, Iran (Bananj *et al*., 2004). It is known as Sar Jamak (local name) in Manujan County in Kerman Province of Iran which blemishes considerable damage to tomato fields each year. Tomato plants become infected in early growth stage, remain dwarf and their size greatly decrease. The leaves that immediately develop after pollution become cup-shaped with deviancy edge to downward, but the leaves which emerge and develop later are deform, chlorosis and small with deviancy edge to upward. Disease symptoms on the fruit depend on the plant age in pollution time.

If pollution occurs in early stages of the plant growth, it loses production ability of marketable fruits. If pollution occurs in the later stage of plant growth, existing fruits consequently are in the natural form but those that arise later are deformed and shed. No outward signs are observed on the flowers but flowers shed are common. Amount of production decrease is related to disease severity and the plant growth stage at pollution time (Zitter, 1991).

In natural conditions, virus transmission is done by insect vector (*Bemisia tabaci*) which is called White Mosquito among farmers of the rejoin; in addition, it can be spread by grafting in laboratory. So far, no reports of transmission have been existed mechanically or via seeds (Zitter, 1991; Bananj and Ahoun Manesh, 1999). The minimum time for virus receiving by vector insects from contaminated plants is 15-20 min and latent period in vector insects is 21 hours. TYLCV virus can be remaining in the vector insects for 10-12 days and rarely more than 20 days. Failure of TYLCV mechanical transmission is an attribute for separating this virus from other viruses which are able to contaminate tomato (Ioannou and Iordanou, 1985). Disease control management of TYLCV is very tricky and costly, and is done by limited factors. Plant disease which caused by viruses are not treatable; therefore, prevention as a strategy for their controlling is considered. Commercial methods for these disease controls are based on vector insects control by exorbitant usage of insecticides or physical methods (such as mantels use with 50 meshes) in infected areas. Other methods such as healthy seedling usage, chemical control of vectors, crop rotation, planting date adjustment, intercropping and resistant varieties are recommended for prevention and damage reduction of TYLCV (Zitter, 1991; Blancard, 1992). Epidemiology of TYLCV and its relation with population density revealed that tomato transplanting led to disease increase and decrease in summer and early autumn (the maximum population of white flies) and winter and early spring (the minimum population of white flies), respectively (Ioannou and Iordanou, 1985). Therefore, different planting date must be investigated for damage reduction of this important crop in South Kerman of Iran.

MATERIAL AND METHODS

A. Climatic characteristics

The project was conducted in South of Kerman (Tejdanu a village in the Central District of Manoujan County) which was pollution center of Sar Jamak (TYLCV). Manoujan with the area of 7500 km is neighbor with Rudan County from the South and West, and with Kahnuj County from the North and East. Geographical location of the county is 57°70' E longitude and 27°44' N latitude. Due to proximity of the county to the sea, it has tropical climate with 59-60 % of humidity at different seasons and height of 337 m above sea level.

The rainfall season start from October to April in Manoujan, and it is under effect of rains and monsoon which derived from Indian Ocean in summertime. The rainfall order has been lost due to drought from 1994 to 2011 and it has been encountered by severe water shortages under continuous drought. The highest prevalence of TYLCV in Kerman province belonged to Manoujan County and it is one of the major problems in tomato cultivation. Therefore, the rejoin was selected for implementation of this project. The project was conducted in the form of randomized complete design with five treatments and three repetitions. Seeds of *Solanum lycopersicum* var. Chef were sown in 5 date (1, 6 August; 2, 21 August; 3, 5 September; 4, 20 September; 5, 5 October) and transplanting to the main land was done in 5 to 6 leaf stage. Each experimental plot had 4 sowing rows with the length of 5 meters. Sowing intervals were considered 45 cm on rows (in zigzag form).

B. Seeds sowing and seedlings transplant

Seedling trays were filled with cocopeat and seeds of *Solanum lycopersicum* var. *Chef* were sown from 6 August with intervals of 15 days. In each sowing date, 105 cell seedlings trays were filled with cocopeat which had been already soaked and 2 or 3 seeds were planted at depth of 1.5 to 2 cm, then were covered with cocopeat and were finally irrigated with wash bottle. Operation care (weeding) was regularly done for all seedlings. Watering, fertility, weeds and other pests control were done without any pesticide.

C. Determination of pollution percent

45, 60, 75 and 90 day after transplanting, it was determined by counting the number of plants with TYLCV disease signs in each plot.

D. Disease severity index

It was done by assessment of plants within each plots, note the date of first appearance of TYLCV signs and calculating disease severity. Score of disease severity was conducted by using Lapidot and Friedman (2002) method as fallow:

0: without outward signs
1: slight yellowing of upper leaf margins
2: mild yellowing and curl of leaflet
3: large yellowing, curl and cup upwards of leaf margins; but the plant was still growing
4: yellowing, severe dwarf, curl, cup upwards of leaf margins and stopping the plant growth

Disease severity index (DSI) was evaluated with following formula for plants within each plots and pollution percent was calculated:

$$DSI = \frac{\Sigma\,(ni.vi)}{n.v} \times 100$$

In the Equation *ni*, *vi*, *n* and *v* shows the plants with the same score, disease score (0-4), total of observed plants and the highest disease score, respectively.

E. Trace molecular

According to biotic and abiotic factors which can cause curl signs in tomato, these signs cannot be cited for disease evaluation; therefore, it is necessary to insure that the signs relate to the presence of virus by using molecular methods. At the moment, molecular methods based on polymerase chain reaction (PCR) by using specific primers are the most accurate, safest and fastest ways to track viruses. Tracking of disease factors in collected samples was conducted by using suitable primers in PCR (Heydarnejad *et al.*, 2009). PCR products were electrophoresed for early identification. In order to determine virus strain, part of PCR product was directly used for determination of nucleotide sequences. Due to different strains of TYLCV, determination of virus strain and genus is so important in this rejoin, it was done by determination of nucleotide sequences/RFLP of PCR product with suitable enzymes.

Obtained data were analyzed by SAS software and means comparison were evaluated by Duncan's multiple range test.

RESULTS AND DISCUSSION

Variance analysis indicated (Table 1) that sowing date significantly affected pollution severity ($\alpha \leq 0.01$). This represents that pollution severity influenced by temperature and fluctuations of insect vector populations. According to lack of alternative hosts and the insect outbreaks in the first to third sowing date, their main activity focused on tomato crops. Whereas, pollution severity and damage extent was notably reduced in the third and forth sowing date.

Means comparison of pollution severity (45 days after transplanting) revealed that there was significant difference between sowing dates. Sowing in 6 August and 5 October showed the highest (52.1 %) and lowest (2.12 %) pollution severity, respectively (Fig. 1). In 60 days after transplanting, the maximum of pollution severity belonged to the second (21 August) sowing date with 74.11 % and sowing in 5 October had the minimum of it with 1.97 %. There was no significant difference between sowing in 6 August and 21 August (Fig. 2). The first and fifth sowing date showed the maximum (82.37 %) and minimum (10.52 %) of pollution severity 75 days from seedling transplanting. While there was no significant difference between the first to third dates (Fig. 3). Investigation of seedlings pollution severity indicated that the highest of it belonged to the first sowing date with 83.52%. The lowest severity of pollution observed in the fifth sowing date with 10.37 %. There was no significant difference between the first to third dates (Fig. 4). It can therefore be concluded that two factors affected pollution severity of pest in the first to third sowing dates. The first one was lack of secondary host which led to increase of the pest focusing on tomato and DSI.

Table 1: Variance analysis of sowing date effect on pollution severity of tomato seedlings.

SOV	df	Mean squares: 45 days after transplanting	60 days after transplanting	75 days after transplanting	90 days after transplanting
Block	2	0.187ns	0.141ns	0.331ns	0.202ns
Sowing date	4	17.27**	21.43**	18.53**	17.903**
Error	8	0.31	0.109	0.317	0.299
CV (%)	-	8.93	5.34	6.97	6.87

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01

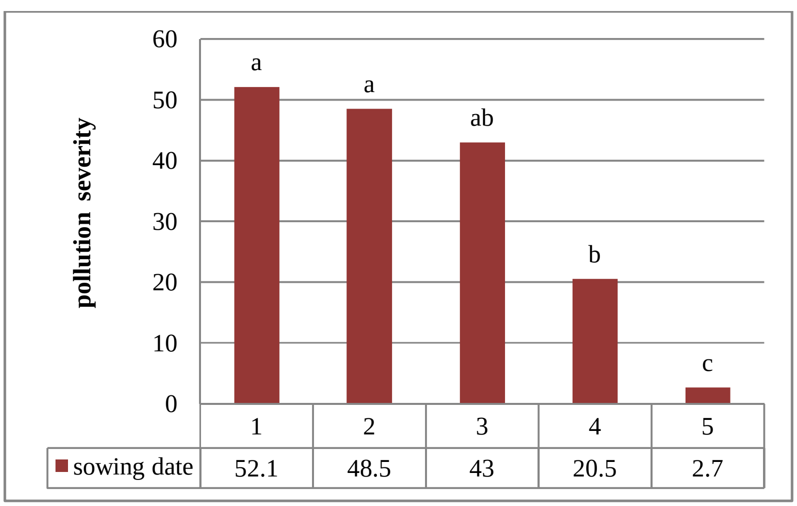

Fig. 1. Seedling pollution severity 45 days after transplanting to the mainland. 1, 6 August; 2, 21 August; 3, 5 September; 4, 20 September; 5, 5 October

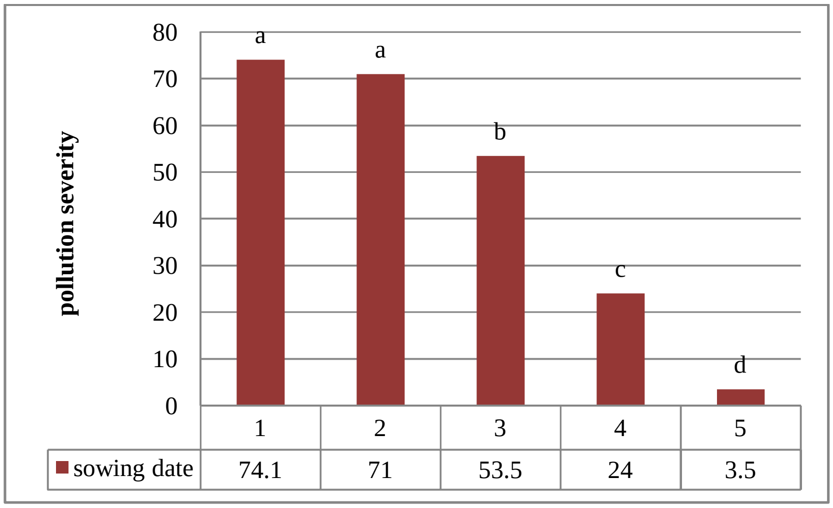

Fig. 2. Seedling pollution severity 60 days after transplanting to the mainland.
1, 6 August; 2, 21 August; 3, 5 September; 4, 20 September; 5, 5 October

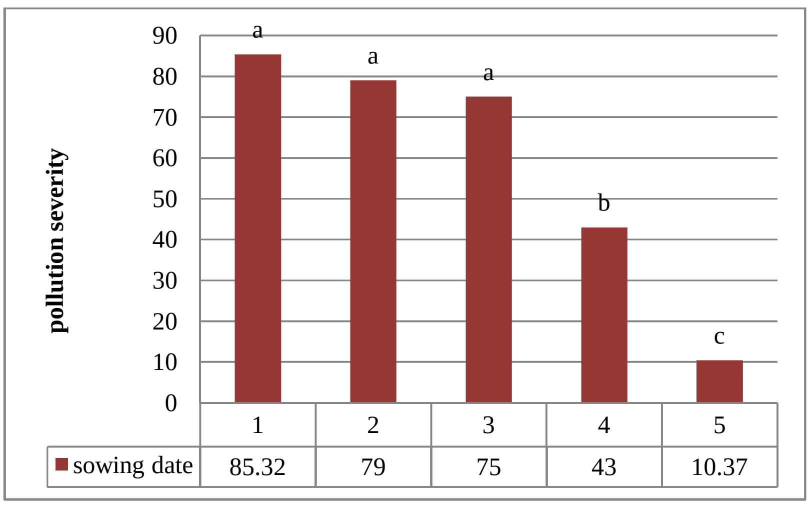

Fig. 3. Seedling pollution severity 75 days after transplanting to the main land.
1, 6 August; 2, 21 August; 3, 5 September; 4, 20 September; 5, 5 October

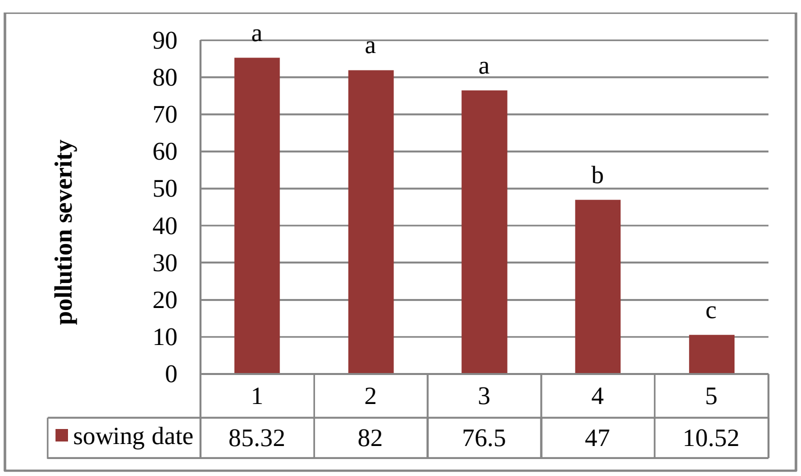

Fig. 4. Seedling pollution severity 90 days after transplanting to the main land.
1, 6 August; 2, 21 August; 3, 5 September; 4, 20 September; 5, 5 October

Outbreaks, faster growth and proliferation of the pest were the second factor. This caused more activity of pest on the crop and increased DSI. Whereas, their population reduced in sowing in 20 September and 5 October due to probability of secondary host and DSI of the main crop was severely decreased.

According to the results (Table 2), sowing date significantly affected pollution percent in all of sampling schedule (α≤0.01). Investigation of seedlings pollution percent indicated that sowing in 6 August had the highest pollution in 45 (Fig. 5), 60 (Fig. 6), 75 (Fig. 7) and 90 (Fig. 8) days from transplanting in the main land with 65.5, 92.3, 97 and 100 %, respectively. According to means comparison results, the lowest pollution percent in 45 (Fig. 5), 60 (Fig. 6), 75 (Fig. 7) and 90 (Fig. 8) days from transplanting in the main land belonged to sowing in 5 October with 1.35, 8.5, 12.5 and 13.2 %, respectively, but no statistically significant difference were observed between the first and second sowing date.

Table 2: Variance analysis of sowing date effect on pollution percent of tomato seedlings.

SOV	df	Mean squares			
		45 days after transplanting	**60 days after transplanting**	**75 days after transplanting**	**90 days after transplanting**
Block	2	0.792ns	0.427ns	0.089ns	0.011ns
Sowing date	4	18.83**	19.97**	17.56**	16.768**
Error	8	0.256	0.239	0.061	0.052
CV (%)	-	8.93	7.51	4.11	3.25

ns, non significant; *, significant at P≤0.05; **, significant at P≤0.01

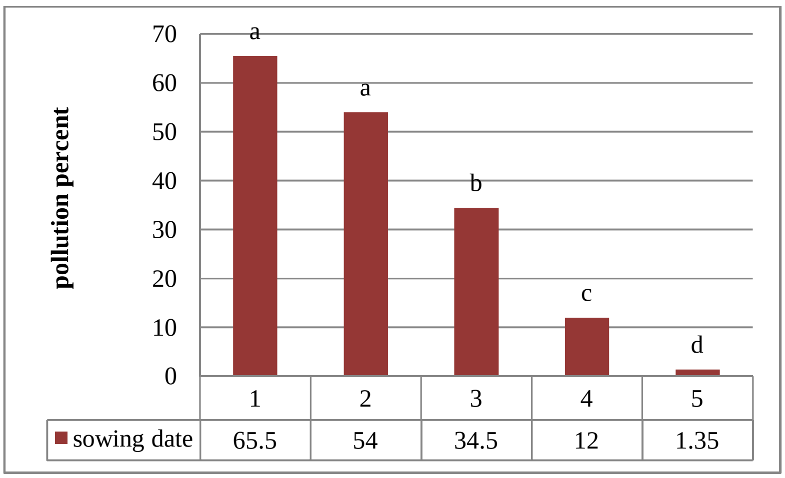

Fig. 5. Seedling pollution percent 45 days after transplanting to the main land. 1, 6 August; 2, 21 August; 3, 5 September; 4, 20 September; 5, 5 October

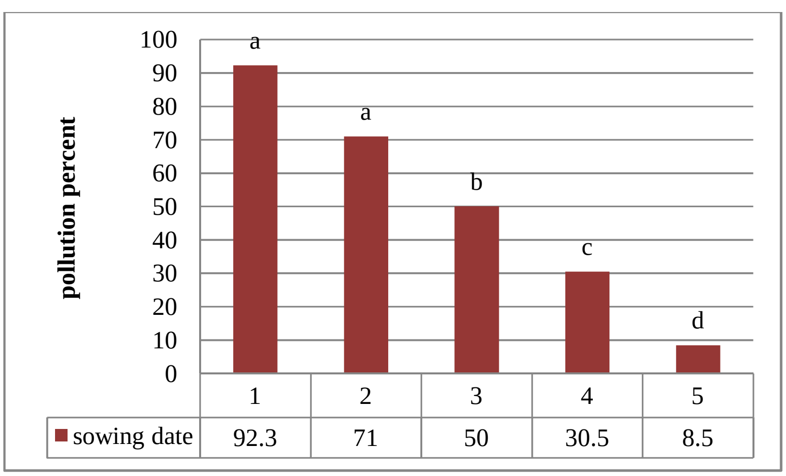

Fig. 6. Seedling pollution percent 60 days after transplanting to the main land. 1, 6 August; 2, 21 August; 3, 5 September; 4, 20 September; 5, 5 October

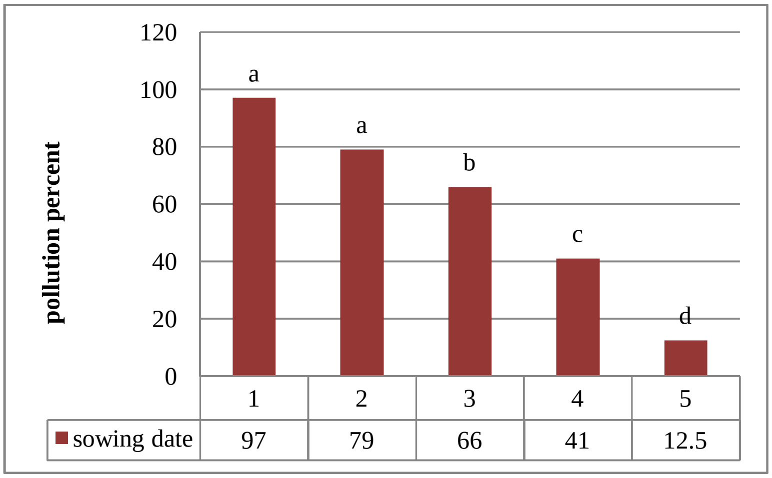

Fig. 7. Seedling pollution percent 75 days after transplanting to the main land.
1, 6 August; 2, 21 August; 3, 5 September; 4, 20 September; 5, 5 October

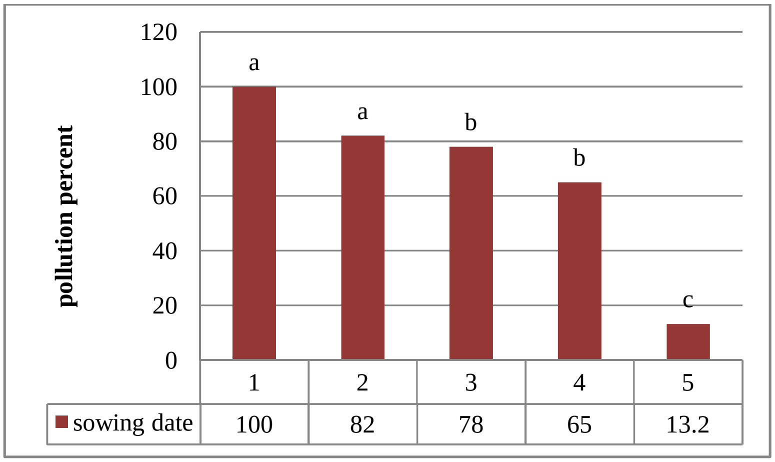

Fig. 8. Seedling pollution percent 90 days after transplanting to the main land
1, 6 August; 2, 21 August; 3, 5 September; 4, 20 September; 5, 5 October

CONCLUSION

Sowing date, as an affecting factor of crop yield and quality, is an important factor in agricultural crops. On the other hand, perceptible changes in climatic conditions have significant effect on plants growth rate and pest population. Due to hot weather, the first to third sowing date led to slow plant growth and pest population growth; therefore, it caused increase in extent of damage to the crops. According to low area under cultivation of the pest hosts in summer, whitefly was more infested tomato crops and damage severity was higher. The lowest amount of damage amount was observed in the fourth and fifth sowing date but price of tomato fruits in 20 September and 5 October was affected by fluctuations in market prices. Although, changing the sowing date in early sowing method was less able to reduce pest damages but using tolerant varieties with proper swing date can reduce damage of Sar Jamak to minimum. Therefore, it is recommended to use tolerant varieties in late August, early September. According to the highest pollution severity in plant nursery stage, seedlings can be protected by insect mesh cover and without spraying, and protect against insect vector. In addition to healthy crop production, it will lead to damage reduction to environment, production and manufacturer revenue increase.

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