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Use of Hot Water-Thermotherapy to Free Potato Tubers of Potato Leaf Roll Virus (PLRV)

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ABSTRACT- The present study was conducted to evaluate the effectiveness of thermotherapy to inactivate the Potato leaf roll virus (PLRV) from the potato tubers. For this purpose, an experiment was carried out at Newly Developmental Farms (NDF) of the University of Agriculture, Peshawar Pakistan. Potato tubers infected with PLRV were collected from farmer's fields. The potato tubers were then treated with hot water at an average 370C for various intervals of time. Afterward, these heat-treated tubers were shifted to fields for sowing. Infield condition minimum % incidence (16.66%) of PLRV was observed from the treatments T3 (2 hours hot water treatment), T4 (2 ½ hours hot water treatment) and T5 (3 hours hot water treatment) respectively while in control 53.33 % incidence of PLRV was recorded. Therefore it can be concluded that thermotherapy at 37^0C for 2 hours, 2½ hours and 3 hours in case of hot water treatment were effective in fully or partially elimination of PLRV from potato tubers. Further, the combined effect of thermotherapy, confidor and neem extract was evaluated against PLRV. It was observed that in T6 (hot water treatment for 2½ hours, insecticide and biocide) % incidence of PLRV was 13.2% with maximum vegetative parameters such as % germination, height (cm), tuber size (cm) and yield (kg) recorded followed by T4 (Confidor + 2½ hours hot water treatment) and T1 (2½ hours hot water treatment), where % incidence of PLRV was 16.66% and 20% respectively. Moreover, the treatment T2 (Confidor), when applied individually, was found to more effective against PLRV as compared to T3 (Neem extract) with an incidence value of 26.66% and 33.33%, respectively.

Key- Words- PLRV, Thermotherapy, Hot water treatment, Confidor, Neem extract

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is herbaceous annual plant belonging to family Solanaceae. It contains about 70% water, 18 % starch, 2% protein while 1% is vitamins, minerals and trace element [1]. It is grown in 140 countries [2].

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Potato is the world's leading food and vegetable crop which originated in South America and now it become important crop in almost all regions including plains of India and Pakistan [3]. The potato was introduced into Europe in 1570 than after fifty years, the potato was brought to Indo-Pak subcontinent from Europe by Portuguese traders. Over the years, potato has become an imperative crop for both farmers and consumers in Pakistan. It is the fourth most significant crop by volume of production; it is high yielding, having a high nutritive value and gives high profits to the farmers. Potato is an important cash and vegetable crop of Pakistan. Three crops of potato are grown

in Pakistan i.e. autumn and spring crops in the plains and summer crop in the hills [4]. Potato is susceptible to various pathogens including fungi, bacteria, nematodes and viruses. Viruses play a vital role for yield reduction in potato crop of Pakistan. Among the viral diseases potato leaf roll virus (PLRV), potato virus Y (PVY), potato mop to virus (PMTV), potato virus X (PVX), potato virus S (PVS) and potato virus A (PVA) are the major viral pathogens have been found throughout the world. As compare to the other pathogens viruses damage plants and cause much more economic losses by reduction in yield and quality of plant products. The severity of individual viral diseases may vary with the locality, virus species, stage of infection and crop variety [5]. Growers introduce high yielding varieties, which enhanced the yield of potato crop but new viral diseases are reported which cause up to 83 % yield losses. Transmission of the viruses by insects is a highly variable process that involves the interactions between the virus, vector and plant [6]. Virus transmission depends on many factors including the aphid biotypes, species, clones, morphs, genotype and virus isolates [7]. Among the plant viruses potato leaf roll virus (PLRV) has been an emerging problem and widely prevalent in all parts of Pakistan [8]. In Pakistan the yield reduction due to PLRV was up to 70% [9]. The yield losses have been recorded up to 90% [10]. Aphid (*Myzus persicae*) is mainly responsible for rapid spread of PLRV in spring potato crop in Pakistan [11]. The effect of the PLRV in potato crop could be manage by integrating three simple concepts: obtaining virus-free certified seed, killing volunteers and weed hosts, and early rouging of infected plants will all help to eliminate primary source of virus inoculums [12]. Efforts have been made to protect crop plants against viruses including potato by producing virus free seed tubers stock, thermotherapy, tissue culture and micropropagation. Pesticides are used to control aphids but many aphid species have become resistant to various chemical compounds. Systemic insecticides and/or accurately timed foliar insecticide applications are useful to reduce within field spread of PLRV, especially if colonizing aphids are virus free on arrival. PLRV was eliminated from diseases tubers of several potato cultivars by hot air as well as hot water treatments [13]. The need to promote more rational use of pesticides has been a great incentive to manage aphids and PLRV. Knowing the importance of potato crop, PLRV and its aphid vectors and by keeping in view of economic situation of farmers the present study is initiated for management of PLRV and its vectors by using thermotherapy alone and insecticide and as a combine treatment.

MATERIALS AND METHODS

Seed tubers of potato cultivar Desiree, commercially grown in Khyber Pakhtunkhwa were collected from Agriculture Research Institute, Tarnab Peshawar in January, 2013. Insecticide (Confidor) and biocide (Neem extract) were purchased from the local market. Tubers of potato cultivar Desiree were treated with hot water. In case of hot water treatment the potato tubers were dipped in water in water bath. The water temperature was maintained at average 37^0C. The tubers were then treated for various times intervals such as 1 hour, 1½ hours, 2 hours, 2½ hours and 3 hours. After hot-water treatment, tubers were dried at room temperature for a week in diffuse light in order to promote sprouting. Two experiments were conducted in Newly Developed Farm (NDF), the University of Agriculture Peshawar. Fields were prepared and afterward cultivation was carried out on ridges using standard agronomic practices. Plants to plant distance were kept 30 cm and row to row distance was kept 60 cm [1]. Under field conditions two experiments were carried out. In the first experiment only heat treated tubers were grown. There were six treatments including control as shown below:

1. 1 hour hot water treated tubers
2. 1½ hour hot water treated tubers
3. 2 hours hot water treated tubers

4. 2 ½ hours hot water treated tubers
5. 3 hours hot water treated tubers
6. Control (Untreated tubers)

In the second experiment efficacy of some insecticides and heat treated tubers were tested and there were seven treatments including control.

1. Heat treated tubers (2½ h at 37 ^{0}C)
2. Insecticides (Confidor)
3. Biocides (Neem extract)
4. Confidor and 2½ h at 37^{0}C
5. Neem extract and 2½ h at 37^{0}C
6. Confidor, Neem extract and 2½ h at 37^{0}C
7. Control

Insecticide (confidor) and Biocide (Neem extract) was applied to a ridge consist of ten potato plants. Insecticides and biocides were sprayed when the aphid population reaches 1-2 aphids/100 compound leaves on the crop and second spraying at 10 to 15 days interval depending upon aphid population. The disease severity of PLRV was assessed on the basis of severity scale as follows;

0 No symptoms

1 Rolling of upper leaves

2 Rolling of upper and lower leaves

3 leaves become stiff and leathery, stunting of plants and erect growth

4 Severe cholorosis, papery sounds of leathery leaves

5 Complete yellowing and stunting, clear rolling of leaves

Percent disease incidence of PLRV was calculated by the following formula:

$$\% \text{ incidence} = \frac{\text{Infected Plants}}{\text{Tested Plants}} x100$$

Data regarding aphid population was gathered by visual count per 100 leaves method at weekly interval, plants were selected randomly through the crop in each ridge. Top, middle and lower parts of plant were examined. The number of Aphids (winged and wingless) was recorded in the following pattern. When the leaf counts show that population of *Myzus persicae* exceeds 20 aphids per 100 leaves, the area was regarded as not suitable for seed tuber production and yield production.

The insecticides and biocides were formulated according to the prescription given with the treatments i.e. for Confidor (Insecticide), recommended dose was 250 ml for one acre, therefore for 800 m^2, 50 ml was used. For biocides (Neem extract), recommended dose was 450 ml for one acre, therefore for 800 m^2, 88 ml was used. When the plant acquired an optimum height and leaves expansion started. Aphid appeared on the potato crop. Soon after the collection of first data of aphid population and virus incidence in the crop, the treatments were applied on respective ridge assigned for the each treatment according to the experimental field layout. All the treatments were applied according to their recommended doses. Seed potatoes were sown on 5th February, 2014. Following the standard agronomic practices, on 5th March, 2014 plants acquired fully expended leaves and up to 6 inches height. First scouting was done for aphids on the same day and then afterward data were collected on weekly basis but the treatments were applied on 10-15 days interval.

RESULTS

Effect of hot water treatments for the inactivation of PLRV in potato tubers

The effect of hot water treatment at 37 ^{0}C for various time intervals for the inactivation of PLRV in potato tubers of cultivar Desiree was determined. The results presented in Table 1 indicated minimum % incidence (16.66%) of PLRV in T3 (hot water treatment for 2 h), T4 (hot water treatment for 2½ h) and T5 (hot water treatment for 3 h). Hot water treatment of potato tubers for 2h, 2 ½ h and 3h at 37 ^{0}C had an apparent influence on PLRV inactivation. Disease severity (DS) level was remained minimum in

these treatments (1) as per scale used. The treated tubers generated plants exhibited mild symptoms such as rolling of upper leaves on 5 plants out of 30 tested plants. Lowest % incidence of PLRV in these two treatments T3 (hot water treatment for 2h) and T4 (hot water treatment for 2½h) justified their superiority to inactivate PLRV as compared to other treatments. Maximum % incidence of PLRV was observed (53.33%) with the disease severity (4) was recorded in T6 (control) where no treatment was applied. In this treatment, severe symptoms such as complete yellowing, stunting of stems and clear rolling of leaves were visually observed on 16 out of 30 tested plants. These results in T6 (control) are indications of highest presence of PLRV in potato tubers as compared to hot water treated tubers. The treatments T2 (hot water treatment for 1½ h) % incidence was recorded 20%. However symptoms such as rolling of upper and lower leaves with Disease severity (2) were observed on 6 out of 30 tested plants. The treatment T1 (hot water treatment for 1 h) with % incidence (26.66%) was superior in performance of PLRV inactivation as compared to T6 (control) with % incidence (53.33%) (Table 1).

Table 1: Effect of hot water treatment at 37^0C for various time intervals for the inactivation of PLRV in potato tubers cv. Desiree

S. No	Treatments	Virus inactivation			Disease severity
		Infected plants	Tested plants	% Incidence	
T1	Hot water treatment for 1 h	8	30	26.66	3
T2	Hot water treatment for 1½ h	6	30	20	2
T3	Hot water treatment for 2h	5	30	16.66	1
T4	Hot water treatment for 2½ h	5	30	16.66	1
T5	Hot water treatment for 3h	5	30	16.66	1
T6	Untreated (Control)	16	30	53.33	4

The combined effect of thermotherapy, insecticide and biocide for the inactivation of PLRV in potato tubers

The combine effect of thermotherapy, insecticide (Confidor) and a biocide (Neem extract) for the inactivation of PLRV in potato tubers is presented in Table 2. Highest % incidence (60%) with severity value (5) was recorded in T7 which was control where no treatment was applied. Severe symptoms such as stunting of stems, yellowing and clear rolling of leaves were observed on 18 plants out of 30 tested plants in T7 (control). On the other hand lowest % incidence (13.2%) with Disease severity (1) was recorded in T6 which was combine treatment of thermotherapy, confidor and neem extract. Moreover just 4 plants out of 30 plants were found infected with mild symptoms such as rolling of upper leaves. Among the treatments lowest % incidence (13.2%) in T6 indicated that it had the maximum potential of inactivation of PLRV from the potato tubers followed by T4 (Confidor + Hot water treatment) and T5 (Neem extract + Hot water treatment) with % incidence 16.66% and 20% respectively. The treatment T2 (Confidor) with %incidence (26.66%) and severity value (3) was found more effective than T3 (Neem extract) with % incidence (33.33%) in individual state for controlling PLRV. Hot water treatment (T1) with % incidence (20%) with Disease severity (DS) (1) was found to be more effective in controlling PLRV then T2 (Confidor) and T3 (Neem extract) with % incidence 26.66% and 33.33% respectively. In the treatment (T1), which is solely hot water treatment, 6 plants out of 30 plants were found to be infected showing symptoms such as rolling of upper leaves.

Hence treatment T1 in its individual state without the application of insecticide and biocide had potential in controlling PLRV in the field conditions. Results exhibited by Table 2 indicated that PLRV was highly affected by combine treatment of thermotherapy, biocide and insecticide as compared to other treatments.

Table 2: Combine effect of thermotherapy, insecticide and biocide for the inactivation of PLRV in potato tubers cv. Desiree

S. No	Treatments	Virus inactivation			Disease severity
		Infected plants	Tested plants	Incidence (%)	
T1	Hot water treatment for 2½ h for 37^0C	6	30	20	1
T2	Confidor	8	30	26.66	3
T3	Neem extract	10	30	33.33	4
T4	Confidor + T1	5	30	16.66	1
T5	Neem extract + T1	6	30	20	2
T6	Confidor + Neem extract + T1	4	30	13.2	1
T7	Untreated (Control)	18	30	60	5

DISCUSSION

PLRV is an economically important virus of potato crop reducing yield and affecting quality of potato tubers [14]. The present research was based on hypothesis, that thermotherapy inactivate PLRV from tubers and symptomology based study was investigated in the field. Though reliable detection techniques such as serology and molecular methods are needed to convince the community of researchers but for poor farmer of Pakistan, thermotherapy of potato tubers to eliminate PLRV and symptomology based study is cheap and cost effective. In susceptible cultivars of potato crop 80% yield reduction by PLRV has been reported [15]. Tuber borne viruses such as PLRV are seriously affecting the potato cultivars of Pakistan.Resistant cultivars of potato are recommended for control of PLRV but it takes several years to develop a new resistant cultivar against PLRV. Despite reports of presence of PLRV in potato cultivars of Pakistan no quarantine measures are undertaken to limit PRLV spread to whole country. Now days there is hardly found a resistant potato cultivar against PLRV. Farmers rely only on insecticides to control aphids to minimize PLRV infection. Insecticide application to control vectors of PLRV also eliminates their natural enemies. Thermotherapy on the other hand is sustainable, cheap and environmentally friendly to get rid of PLRV from potato tubers since its introduction by [16]. Regarding the thermotherapy it was observed that hot water treatment of potato tubers at 37^0C for 2 hours 2½ hours and 3 hours with minimum % incidence (16.66%) of PLRV were found to be effective in reducing PLRV from potato tubers whereas in case of hot air treatment of potato tubers at 37^0C for 3 hours with minimum % incidence was regarded best as compared to other hot air treatments in inactivation of PLRV from potato tubers. Similar effect of thermotherapy on disease suppression has been reported to produce clean cassava planting materials from cassava mosaic virus [17]. Maximum % incidence (53.33%) was recorded from the control which consists of untreated tubers. In our study the assumption of PLRV inactivation from potato tubers was associated with the reduction of % incidence of PLRV under field conditions. The minimum % incidence (16.66%) in potato crops grown from the tubers which were treated with hot water at 2 hours, 2½ hours and 3hours indicated reduction of PLRV and the viruses such as alfalfa mosaic and tomato black ring spot virus from the tubers through thermotherapy.[18] Achieved highest

percentage of PVY eradication with the values 90%, 93% 93.4% and 96% for cv. Desiree, Cardinal, Diamant and Sante, respectively through thermotherapy. The plants which were subjected to thermotherapy heavily reduced viral nucleic acid as reported by the researcher [19], who applied thermotherapy to inactivate raspberry bushy dwarf virus (RBDV) from the raspberry seeds. Similar findings of low % incidence were achieved by [20]], who found 30% reduction of PVY after treatment of potato plantlets with thermotherapy at 37^0C for various interval of time. Percent incidence of PLRV as well as severity was reduced when tubers were treated with thermotherapy. Thermotherapy applied to potato tubers had been successfully used as standard criteria for inactivation of many potato viruses. For most plant cultivars, thermotherapy is usually done at 37^{0}C, however, the exact temperature and length of treatment varies with the virus and genotype of the plant [21]. [22] obtained 60% reduction of PLRV from the potato tubers of cv. Cardinal. They reported 60% elimination of these viruses when meristem culture alone was used but when combined with thermotherapy 100% elimination was achieved. Hussain *et al.* [23] eradicated peanut mottle virus (PeMoV) from 24% of peanut plants. Virus eradication indicated further support for the use of thermotherapy as the best treatment for virus elimination in potato [24]. In our findings minimum % incidence in potato plants grown from hot water treated for 2 hours, 2½ hours and 3 hours are also in concordance with the results of [25], who observed similar results, reducing the concentration of PVY, PVX and PVS, when plants were submitted to thermotherapy. In contrast to the present experiment, the researcher [26] applied same interval of time but with three levels of thermotherapy (27^0C, 30^0C and 37^0C) to eliminate PLRV from the meristem of potato crop. Ali *et al.* [27] reported eradication of plum pox virus (PPV) at 37^0C to 39^0C for 2½ hours interval of time. Moreover researchers had applied hot water treatment against sweet potato feathery mottle virus (SPFMV) and his study results revealed that viability of few sweet potato tubers were suffered when time interval of hot water treatment was increased. Same experiments on elimination of plant viruses through thermotherapy were conducted by the researcher [28]. Qu *et al.* [29] reported that hot water treatment of potato tubers for longer duration can reduce viability of potato tubers. These findings that 2 hours, 2½ hours and 3 hours hot water treatments and 3 h hot air treatments of potato tubers at 37^0C raised a question of the mechanism by which thermotherapy eliminate PLRV from potato tubers and thus reducing its incidence (%) in the field. Previous literatures revealed that thermotherapy inhibits viral replication and increase its degradations inside plant tissues. Efficiency of virus inactivation through thermotherapy in a given host varies depending on the virus strain and host genotype [30]. Furthermore In our findings that combine spray application of confidor and neem extract over potato crops grown from hot water treated tubers at 37ºC for 2½ hours time interval were effective in reducing % incidence of PLRV in field conditions with a value of 13.2% as compared to other treatments. In case of individual spray of confidor and neem extract % incidence of PLRV was reported 26.66% and 33.33% respectively. In individual state neem extract was less effective in minimizing PLRV% incidence than confidor. Beemster and Rozendal [31] stated that insecticide control *M. persicae* consequently preventing PLRV transmission to potato crop. Foster *et al.* [32] reported reduction of PLRV incidence by spraying different insecticides against *M. persicae* which is the most efficient vector of PLRV. Insecticides may strongly minimize virus spread from plant to plant within the field. Moreover applications of biocides and insecticides are recommended on arrival time of aphids, crop age and aphid biology.

CONCLUSIONS

In conclusion, thermotherapy (hot water at 2-3 hours at 37^0C) is found to be effective for the inactivation of PLRV from the potato tubers of cultivar Desiree. It can also be

concluded that combination of thermotherapy (hot water at 2 ½ hours at 37^0C) as separate and thermotherapy with combination of insecticide (Confidor) are observed to be effective in management of PLRV from potato tubers.

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