https://doi.org/10.56093/ijas.v90i7.105616

Short Communications

Indian Journal of Agricultural Sciences **90** (7): 1345–7, July 2020/Short Communication

Decontamination of cyst (*Globodera* spp.) infested potato seed tubers with sodium hypochlorite (NaOCl)

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Received: 13 March 2019; Accepted: 09 October 2019

Key words: *In vitro, In vivo,* Potato cyst nematode, Sodium hypochlorite, Tuber treatment

In India, PCN (*Globodera* spp.) was first detected in 1961 from a field in Vijayanagaram farm in Udhagamandalam, Nilgiris district, Tamil Nadu (Jones 1961). Recently, PCN has been intercepted from some parts of Himachal Pradesh, Jammu & Kashmir and Uttarakhand hills as a result Government of India restricted the movement of potato seeds from the infested areas of these states to rest of the country. Therefore, quarantine has been imposed in many countries to restrict the movement of seed potato tubers in order to prevent its further spread to non-infested areas. Washing and brushing of the tubers is very efficient for successful removal of PCN cysts from potato tubers grown in sandy loam soil heavily infested with PCN (Karanastasi and Kormpi 2011). However, washing and decanting of water will increase the PCN population in the particular area. Sodium hypochlorite (NaOCl @1%) solution disinfects potatoes by destroying the PCN cysts and eggs in 30-45 min. (Wood and Foot 1977). NaOCl is also a well known bleaching agent and disinfectant, the compound in solution is unstable and decomposes easily by liberating chlorine. It is effective against bacteria, viruses and fungi and is being used as sterilizing agents in food industries (https://en.wikipedia.org/wiki/Sodium_hypochlorite). Therefore, an experiment was conducted to study the effect of NaOCl treatment on PCN and potato seed tubers infested with PCN during 2019 at ICAR-CPRS, Kufri, Shimla and ICAR-CPRS, Muthorai, Udhagamandalam. Accordingly, effect of different concentration of NaOCl (4% available chlorine) lab grade (RANKEM NaOCl Solution 4%) was evaluated under *in vitro* condition on PCN disintegration. Different concentrations (v/v) of NaOCl, viz., T_1- NaOCl @0.5%; T_2- NaOCl @1.0%; T_3- NaOCl @2.0% and T4-Water (control) were evaluated in a completely randomized design with five replications. Required concentration of NaOCl was prepared by diluting required quantity of NaOCl 4% solution in water. To check the effect of NaOCl on cyst disintegration, 200 cysts were kept in sterilized petridish and 50 ml of each dilution was added and incubated in room temperature. Observation on cyst disintegration was taken after 10, 30, 60, 90 and 120 min. after incubation. The efficacy of NaOCl on cyst disintegration was recorded with the help of stereo zoom microscope and expressed in per cent. Hatching efficacy of left out non-disintegrated eggs was also checked in root leachates after seven days of incubation.

Effect of different concentrations of NaOCl was evaluated under *in vivo* condition using PCN infested tubers. The experiment was carried out with treatments of different concentrations of NaOCl with different duration of dipping, *viz*., T_1-NaOCl @0.5% (30 min); T_2-NaOCl @0.5% (60 min); T_3-NaOCl @0.5% (120 min); T_4-NaOCl @1.0% (30 min); T_5-NaOCl @1.0% (60 min); T_6-NaOCl @1.0% (120 min); T_7-NaOCl @2.0% (30 min); T_8-NaOCl @2.0% (60 min); T_9-NaOCl @2.0% (120 min); T_{10}-Water (30 min); T_{11}- Water (60 min); T_{12}- Water (120 min); T_{13}- Boric acid @3.0% (30 min) in a completely randomized design with five replications. Required concentration of NaOCl was prepared by diluting of NaOCl 4% solution in water. Potato tubers collected from the field infested with PCN were used in the study. Ten tubers per replication were dipped in the beaker containing 1.5 litre of different concentration of NaOCl along with control. Observations on numbers of cysts floated/dislodged were taken after treatment period after passing through the solution on 840 µm and 250 µm sieves. The tubers and soil debris remained on 840 µm sieve and cysts were retained on 250 µm sieve during rinsing and were counted. After rinsing the tubers were shade dried and stored in gunny bags for two months to check the effect of different treatments on sprouting of tubers.

Effect of sodium hypochlorite (NaOCl) on PCN

Our results showed that 100% cyst disintegration under *in vitro* condition was recorded in NaOCl @1% after 30 min. of incubation (Fig 1), whereas under *in vivo* condition, it took

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60 min. to disintegrate 100% PCN. At higher concentration (NaOCl @2%), Cent percent cyst disintegration under *in vitro* and *in vivo* conditions occurred after 10 and 30 min. respectively. It was reported that, the germicidal activity of a concentrated sodium hypochiorite solution is based on its high pH (-OH action) and -OCl oxidation (Estrela *et al.* 2002) and higher concentration is more aggressive (Holland *et al.* 1992). HOCl and -OCl have also been reported to react with biological molecules such as proteins, amino acids and peptides (Hawkins and Davies 1999; Nightingale *et al.* 2000). The time taken for cyst disintegration under *in vivo* condition is comparatively more as compared to *in vitro* condition and this may be due to presence of soil particles adhere in seed potato tubers which contain organic matter as reported by Wood and Foot (1977). Grigg and Chase (1967) also used chlorine solution for surface disinfection of potato tubers. Similarly, disintegration of soybean cyst nematode with NaOCl has also been reported (Esser 1972). In addition to PCN disintegration, the lustre of seed tubers was also improved after treatment with NaOCl as compared to water and boric acid. The results showed that there was no deleterious effect on tuber sprouting due to NaOCl treatment. Wood and Foot (1977) also mentioned similar non-toxic effect due to NaOCl on plant. However, Gardner *et al.* (2006) reported phytotoxic and corrosive effects of NaOCl on potato tubers. Manoharan *et al.* (1978) reported use of calcium hypochlorite (9%) for reducing PCN field populations through seed treatment. In our study, we directly used the harvested tubers for treatment without initial washing with water. However, Wood and Foot (1977) initially washed the infected tubers with water before treatment. They showed that 1% NaOCl solution disinfected potatoes by destroying the PCN cysts and eggs in 30-45 min. Their work was successfully replicated to disinfect seed potato tubers from cysts of PCN (Brzeski and Rogala 1984).

Efficacy of NaOCl on seed tubers infected with PCN

PCN infested seed potato tubers were treated with different concentration of NaOCl, *viz.* 0.5, 1.0 and 2.0% for different durations, *viz.* 30, 60 and 120 min. to check its effect on PCN disintegration along with control. The results showed that increase in concentration reduced the time required for disintegration of cyst wall. Among the treatments, 100% floating and settled cyst disintegration was recorded in NaOCl @2.0% (T_7) after 30 min., 60 min. in NaOCl @1.0% (T_5), whereas it took 120 min. in NaOCl @0.5% (T_3). While in control and boric acid @3.0% (T_{13}), there was no disintegration of cysts (Table 1). In addition different treatments had no adverse effect on sprouting of seed tubers after two months of storage.

Cost of treatment

Five litre of NaOCl 4% (Lab grade) is required to treat 1.25 quintal of seed tubers. The cost of laboratory grade NaOCl comes to ₹ 100/litre. Average cost of chemical required to treat 1.25 quintal of seeds by NaOCl through dipping is around ₹ 500. The ISI Grade-1 NaOCl (4%)

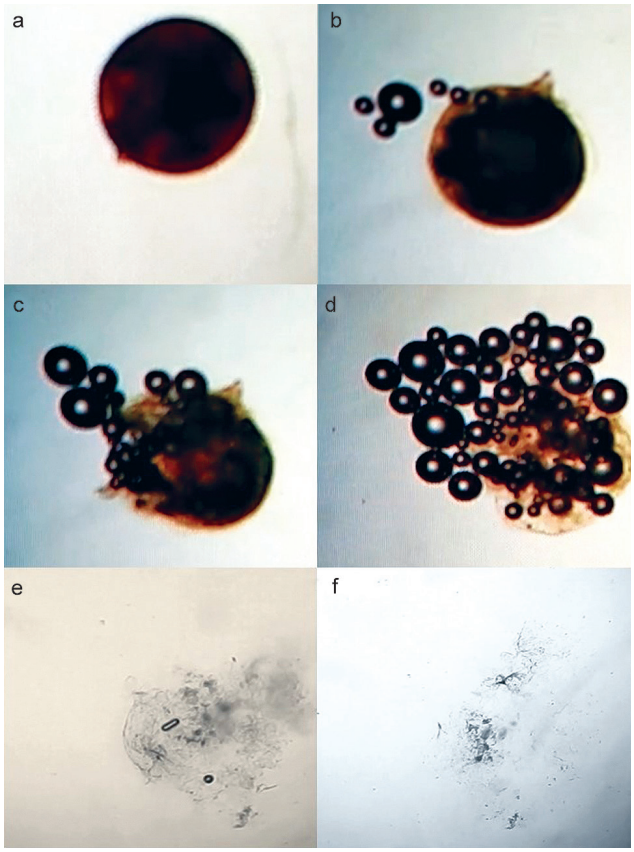

Fig 1 Disintegration of cyst in NaOCl 2% solution. a. Intact cyst; b. Initiation of cyst wall disintegration; c. Partially disintegrated cyst; d. Completely disintegrated cyst; e. Partially disintegrated eggs; f. Completely disintegrated eggs

Table 1 Effect of NaOCl on PCN infested harvested tubers

Treatment		PCN population (cysts)			Seed tuber sprouting (%)
		Initial floating	Final floating	Final settled	
T_1	NaOCl @0.5% 30 min.	18	0	8	100
T_2	NaOCl @0.5% 60 min.	21	0	6	100
T_3	NaOCl @0.5% 120 min.	19	0	0	100
T_4	NaOCl @1.0% 30 min.	17	0	5	100
T_5	NaOCl @1.0% 60 min.	18	0	0	100
T_6	NaOCl @1.0% 120 min.	17	0	0	100
T_7	NaOCl @2.0% 30 min.	16	0	0	100
T_8	NaOCl @2.0% 60 min.	19	0	0	100
T_9	NaOCl @2.0% 120 min.	20	0	0	100
T_{10}	Water 30 min.	10	10	16	100
T_{11}	Water 60 min.	15	15	19	100
T_{12}	Water 120 min.	18	18	15	100
T_{13}	Boric acid @3.0% 30 min.	24	24	9	100
	SEd	2.29	1.40	1.02	
	CD (P=0.05)	4.70**	2.87**	2.09**	

also gives comparable results as that of laboratory grade. Accordingly, the cost will be reduced to ₹ 300 for treating 1.25 quintal of seeds by dipping. The results showed that the solution once prepared can be used for soaking the seed tubers 12 times for a period of 30 min. per soak without affecting the efficacy on cyst degradation. Even after twelve times if the farmer wishes to use the solution further, the soaking time is to be increased from 30 to 40 min.

Potato cyst nematode is a quarantine pest worldwide, therefore, disinfection of seed tubers before transporting to non-infested area is must. Hence, treating the tubers infested with PCN after harvest using NaOCl 2% can disintegrate the cysts on potato with in short period (30 min.) without affecting the seed tuber quality and will also help in avoiding spread of PCN through seed tubers. However, the effect of decanting left out NaOCl solution after soaking treatment on soil micro flora needs to be studied.

SUMMARY

Potato cyst nematode (*Globodera* spp.) is one of the serious pests of potato worldwide including India. Infested seed tubers and soil are the primary means of spread of cysts to non-infested areas, whereas air, water, human, animals, field equipments, composts, gunny bags are other means of spread. Therefore, a study was carried out with different concentrations of NaOCl under *in vitro* and *in vivo* condition to see its decontamination effect on potato cyst nematode (PCN). The results revealed that potato tubers harvested from the PCN infested fields can be completely decontaminated by giving dip treatment in NaOCl (2%) solution for 30 min. and the treated tubers sprouts normally after two months of storage.

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