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Validation of farmer's practice of using sodium chloride for controlling foot rot disease of black pepper (*Piper nigrum*)

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ABSTRACT

An innovative field observation by a farmer indicated that common salt (sodium chloride) is effective in controlling *Phytophthora* foot rot of black pepper (*Piper nigrum* L.) caused by *Phytophthora capsici*. This is validated through a series of *in vitro* and *in vivo* experiments. Initially mycelial growth, sporangial formation and zoospore germination of *P. capsici* were studied on a range of concentrations from 0.01 M to 3.0 M of NaCl. It was found that mycelial growth is inhibited by 1 M, Sporangial production by 0.75 M and zoospore germination by 0.5 M sodium chloride respectively. But, *in vivo* studies by challenge inoculation with *P. capsici* showed that the maximum inhibitory concentration under *in vitro* (1 M) is insufficient to inhibit *P. capsici* in the soil. Hence, higher concentrations, viz 2 M to 8 M were tested in soil. The results showed that 3 to 4 M concentrations of sodium chloride are the maximum required to destroy the soil inoculum but was found phytotoxic. Modification of the treatment by washing-off the soil amended with salt resulted in nullifying the phytotoxic effect without affecting the total microbial biomass, nutrient status, *p*H or electrical conductivity of the soil. This method can be used as a pre- planting practice while rejuvenating a diseased garden or while gap-filling or while raising nursery plants in potting mixture.

Key words: Black pepper, Electrical conductivity, Foot rot, *Phytophthora capsici*, Phytotoxicity, Rejuvenation, Sodium chloride

In black pepper (*Piper nigrum* L.), crop loses due to diseases and pests are major production constraints. In India, Indonesia and Malaysia, *Phytophthora* foot rot is the major disease reported in black pepper. *Phytophthora* infections in black pepper are broadly classified into aerial and soil infections. The aerial infection is manifested as aerial infection on leaves, spikes and runner roots, whereas soil infection resulted in root rot and collar rot which will be recognized only at a later stage as foliar yellowing or quick wilt.

An integrated disease management strategy involving cultural, chemical and biological method is recommended for the effective management of the disease (Anandaraj 2000) which includes *Trichoderma* and *Pseudomonas fluorescens* (Rajan 2000). Besides, there have been several reports where inorganic salts have been used for controlling pathogens under *in vitro* and *in vivo* (El-Shami *et al*. 2004). Similarly, role of certain eradicative fungicides that are toxic to plants but useful for suppressing *Phytophthora* in soil and water and on glasshouse benches were also in practice. In this study, a farmer's experience of using common salt for effective control of spread of foot rot disease of black pepper has been validated. The farmer has claimed that he could manage the disease by merely applying common salt. Based on the report, the authors closely observed his plants treated with sodium chloride and found that the treated plant gets killed due to salt treatment, however, there was no spread of the disease to nearby plants. Since the indigenous technologies forms part of indigenous technical knowledge (ITK), in this study, these technology is validated through a series of experiments and modified it as an acceptable technology.

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MATERIALS AND METHODS

The experiment was conducted during 2006–08. In the first phase the effect of different molarities of NaCl were tested *in vitro* against different growth stages of *P. capsici*. In the second phase, its effect was studied *in vivo* on soil plant environment by inoculation and in the third phase the technique was improvised and tested with host.

Different molar solutions of NaCl, viz 0.01 M to 3.0 M were prepared in distilled water. *P. capsici* isolate 05–7 isolated from collar rot affected plant was used for the studies.

Three-month-old black pepper cuttings raised in sterile potting mixture (sterilized by solarization by keeping the moistened potting mixture in hot sun for 40–45 days) was used for *in planta* studies.

For *in vitro* studies, different concentrations of NaCl (0.01 M, 0.1 M, 0.25 M, 0.50 M, 0.75 M, 1.0 M, 1.5 M, 2.0 M, 2.5 M, and 3.0 M) were incorporated in carrot agar(CA), sterilized at 121°C for 20 min. poured into Petri-dishes and inoculated with 5 mm discs of 72 hr-old actively growing culture of *P. capsici* in triplicate and incubated at 24±1°C in the dark for 72 hr. Control was kept with *P. capsici* alone. The colony diameter was measured after 72 hr.

For sporulation, mycelial plugs of 5 mm size taken from the edge of 72 hr-old culture and incubated in different concentrations of NaCl under light. Also maintained a control in distilled water. The number of sporangia formed/disc was microscopically counted after 48 hr (Biles *et al.* 1995, Zhang, 1988).

Similarly, for germination studies, mycelial plugs as above were allowed to sporulate in different concentrations of NaCl. After 48 hr, the sporulated discs were subjected to cold shock treatment for 10 min. to release the zoospores. From this released zoospores, 1 ml each was added to 5 ml of different concentrations of NaCl and allowed to germinate keeping zoospore solution in 5 ml of distilled water as control. The zoospore germination was counted after 2–3 hr.

The effect of different concentrations of NaCl, viz 1 M, 2 M, 4 M and 6 M on soil inoculum of *P. capsici* was studied based on *in vitro* experiment. Sterile soil was taken in disposable cups, moistened, and inoculated with 5 numbers of 10 mm discs of *P.capsici* and incubated for 5 days and then treated with different concentrations of NaCl and incubated for 72 hr. Three replications were maintained for each concentration along with control in sterile soil inoculated with *P.capsici* alone. After 72 hr, 50 g of the soil was sampled and assayed for *Phytophthora* population by baiting using *Albizzia falcataria* leaf lets.

In planta effect was studied using 3-month-old rooted cuttings of black pepper raised in polyethylene bags of size 25 cm × 15 cm. Initially, these plants were inoculated with 5 numbers of 10 mm discs of *P.capsici*. After 72 hr, these plants were treated with different concentrations of NaCl, viz 1 M, 2 M, 4 M, 6 M and 8 M. Five replications were maintained for each concentration maintaining a control without NaCl treatment and observations were recorded daily.

Based on the results of the *in planta* experiment above, another experiment was laid out with complete randomized block design to nullify the phytotoxic effect with 9 replications consisting of 6 treatments including control. The treatment were, 5 different concentrations, i e 2 concentration below maximum inhibitory concentration and 2 concentrations above inhibitory concentration of NaCl, viz 2 M, 3 M, 4 M, 5 M and 6 M. Earthen pots of size 30cm × 30cm were filled with approximately 16 kg of potting mixture (1:1:1 sand, soil and cow dung) and moistened. These pots were inoculated with *P. capsici* (one 90 mm plate culture grown in carrot agar for 7days, macerated and added to each pot). After 20 days of inoculation, the pots were treated with different concentrations of NaCl @ 500 ml/pot. Five days after treatment, the pots were daily leached with water to remove the traces of NaCl and continued for 10 days. These pots were then planted with 3-month-old rooted cuttings and observed for mortality and infection. Simultaneous with this, the *Phytophthora* population was estimated by baiting at every step, ie before and after NaCl application and after leaching for 10 days. So also the soil was analyzed for *p*H and EC after imposition of treatments and after leaching, and the soil was analyzed for N, P, K, Ca, Mg and Na. The soil was also analyzed for estimating the total fungal and bacterial population.

Since *Trichoderma* species are being used to control foot rot disease in black pepper, an *in vitro* bioassay was also done with the inhibitory concentration of NaCl (1 M) against *T. harzianum* by poisoned food technique. Potato Dextrose Agar medium incorporated with different concentration of the salt were sterilized, poured in Petri-dishes and inoculated with 5 mm discs of 72 hr old actively growing culture of *T. harzianum* and incubated at 24°±1°C for 72 hr. Control was kept with *T. harzianum* alone without the addition of salt. There were 3 replications. The diameter of the colony was measured after 72 hr.

Effect of the maximum inhibitory concentration of NaCl on total fungi and bacteria were also estimated from the treated soil after leaching with water using soil dilution plating in Martin's Rose Bengal Agar (RBA) and Nutrient agar (NA) respectively for fungi (10^{-5}) and bacteria (10^{-6})

RESULTS AND DISCUSSION

Effect of different concentrations of NaCl on *P. capsici* under *in vitro* conditions showed that 1 M is completely inhibitory to mycelial growth. The highest growth was observed in 0.01 M (75.7 mm) and is very much comparable to control (74.64 mm) The least growth was observed in 0.75 M (7.5 mm) (Table 1).

Sporulation was also found inversely proportional to concentration. Maximum inhibition of sporangial formation was noticed in 0.75 M and above when compared to 0.01 M and untreated control where the number of sporangia produced/microscopic field is around 250, followed by 0.1 M (91–123), 0.25 M (49-82) and 0.5 M (0–0.4 sporangia/mf) (Table 1). But when zoospore germination was observed, 0.5 M showed maximum inhibition, followed by 0.01 M (94%) and 0.1 M (99%) (Table 1). So, from the overall *in-vitro* study, it is found that 1 M sodium chloride is inhibitory to all the developmental stages of *P. capsici*.

When different concentrations of NaCl from 1 M to 8 M were tested on soil inoculum of *P.capsici*, it was found that, up to 2 M concentration is insufficient to inhibit the fungus

Table 1 *In vitro* effect of sodium chloride on mycelial growth, sporulation and zoospore germination of *P. capsici*

Treatment	Growth diameter(mm)	Sporulation spores/microscopic field	Zoospore germination (%)	Inhibition of zoospores over control (%)
Control	74.67	249.75	48.83	
0.01 M	75.5	218.33	2.88	94.10
0.1 M	63.17	107.40	0.36	99.26
0.25 M	46.83	64.73	0.40	99.18
0.50 M	27.50	0.20	0.0	100.00
0.75 M	7.50	0.0	0.0	100.00
1.0 M	0.0	0.0	0.0	100.00
2.0 M	0.0	0.0	0.0	100.00
3.0 M	0.0	0.0	0.0	100.00
CD (*P*=0.05)	1.208	20.145	2.748	

Table 2 Variation in electrical conductivity (dS/m) and *p*H

Treatment leaching	Concentration	EC before leaching	EC after leaching	*p*H before leaching	*p*H after leaching
T_1	(2 M)	10.2	0.19956	5.364	6.573
T_2	(3 M)	11.289	0.16400	5.508	7.061
T_3	(4 M)	14.778	0.21444	5.702	7.161
T_4	(5 M)	19.744	0.23578	5.543	7.038
T_5	(6 M)	20.133	0.29211	5.610	7.127
T_6	Control	0.367	0.13356	6.487	6.689
CD (*P*=0.05)		2.6688	0.0335	0.1544	0.1401

in the soil (Table 2). More than 96% bait infection was obtained in 1 M and 2 M, but 4 M onwards showed complete inhibition of the soil inoculum (Fig 1).

When 3-month-old plants raised in polyethylene bags were inoculated with *P.capsici* and treated with different concentrations of NaCl, viz 1 M, 2 M, 4 M, 6 M and 8 M, the plants got wilted with in 24 hr and died subsequently due to phytotoxicity, whereas the plants in the control took up infection in 7–9 days after inoculation and died.

The experiment for nullifying the phytotoxic effect was done based on *in planta* results shown above. The disease potential index (DPI)of the soil at each step of estimation is represented in Fig 2. The DPI of the soil before treatment was 32 and after treatment, the DPI lowered down to zero (2 days after treatment).When these pots were leached with several changes of water for 10 days, and then baited, no infection could be obtained on the baits from 3M concentration and above (Fig 2) when compared to control where the DPI become 64. So also no phytotoxic symptoms could be noticed. The control plants started dying from the day 9 onwards due to *P. capsici* infection and all the plants died in 20 days (Fig 3). 2 M concentration showed only very negligible mortality due to *Phytophthora* infection, whereas in other concentrations not even a single plant got infection till the end of the experimental period of 3 months. Here, in this experiment, even 3 M concentration is found inhibitory to soil inoculum of *P. capsici*. The result of this experiment

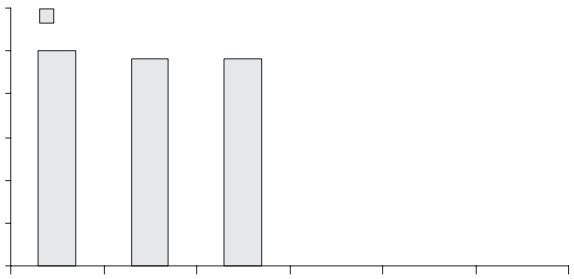

Fig 1 Per cent bait infection by *P. capsici* in soil at different concentration of NaCl

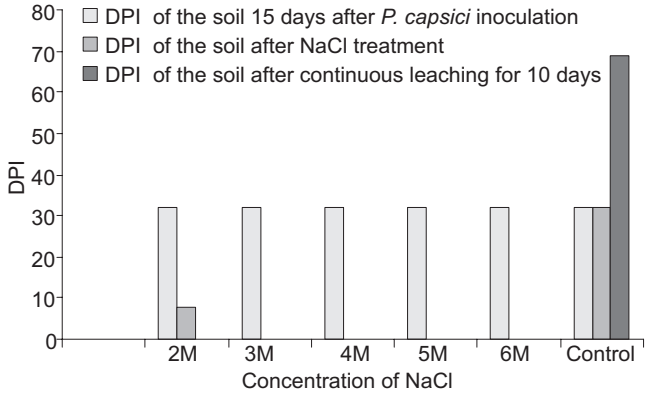

Fig 2 Disease potential index of the soil before and after application of different concentration of NaCl

Table 3 Nutrient level (ppm) of soil in various treatments after flooding

.Treatment	Concentration	N	P	K	Ca	Mg	Na
T_1	(2 M)	163.33	216.67	458.30	1295.70	417.70	314.00
T_2	(3 M)	146.67	205.00	404.70	1060.00	393.30	263.000
T_3	(4 M)	151.67	227.67	390.30	1012.70	401.30	250.33
T_4	(5 M)	157.33	207.33	428.70	1105.00	334.30	318.33
T_5	(6 M)	158.33	176.67	385.70	742.70	388.70	310.00
T_6	(Control)	170.00	208.33	515.70	1332.70	412.30	58.67
	CD(*P*=0.05)	34.2476	16.2181	60.5230	201.6929	102.8787	111.2513

clearly indicated that leaching helped in reducing the salt content of the soil and thereby nullified the phytotoxic effect.

The soil was tested for *p*H and EC after application of different concentration of NaCl and 10 days after leaching. Application of NaCl reduced the *p*H of the soil to acidic status but it is recouped to normal after thorough leaching. Similarly, the electrical conductivity has gone up to 20.133 from 0.367 in the highest concentration (6 M), but after thorough leaching for 10 days, the electrical conductivity has gone down to 0.29211 and corresponding decrease was noticed in all the concentrations. Hence, the results showed that electrical conductivity and *p*H can be brought back to normal by reducing the salt content through leaching (Table 2).

There is no significant change in nitrogen content in treated and untreated soil. Similarly, significant difference was also not observed in phosphorus content except in the higher concentration (6 M) where it is found significantly lower than in any other concentration when compared to control. The phosphorus content in other concentrations was at par with control, indicating that the inhibitory concentration of salt is not affecting the P content of the soil. But, there is a reduction in potash content which is significantly lower than that in control. The potash content was 404.70 in 3 M and 390.30 in 4 M as against 515.70 in control (Table 3). There is a significant increase in sodium content in all the treatments when compared to control while there was no significant difference with Mg content. But, there was a decrease in Ca content with increasing concentration of NaCl (Table 3). This is in accordance with the work done by Chatzissavvidis *et al*. 2008. He also reported that salinity had no effect with regard to P, Fe, Mn and Zn concentrations, whereas the K concentrations of the leaves increased. Yagmur *et al*.(2007) found a negative effect of NaCl on the growth of wheat seedlings by decreasing the emergence percentage, dry shoot and root weights, shoot K +, Na + ratio, chlorophyll a and b content and osmotic potential. However, it increased proline concentration compared to non-salinity conditions (Yagmur *et al*. 2007)

The inhibitory concentration was also tested for its effect on *Trichoderma harzianum* (IISR 1369) which is being used as the biocontrol agent against *P.capsici* in controlling foot rot disease of black pepper. Though, there was an initial reduction in the growth of *Trichoderma*, it resumed growth gradually and filled the entire plate within 5 days, when compared to the control where full growth in plate was obtained within 3 days (Fig 4). The result showed the adaptability of *Trichoderma* with saline conditions. This is supported by the work of Elmer. He combined non-pathogenic strains of *Fusarium oxysporum* with NaCl to suppress *Fusarium* crown rot of asparagus in replanted fields (Elmer 2004). Similarly, no significant difference in total fungal or bacterial population was obtained in 3 M or 4 M concentrations (Fig 5) when compared to control, indicating that the salt treatment is more or less specific to the pathogen.

Results of the two pot culture experiment revealed that 4 M is the maximum concentration required to kill the pathogen in the soil. When these concentrations were applied to the soil in presence of the host, i e black pepper, the plants wilted within 24 hr of application which shows the phytotoxic effect.

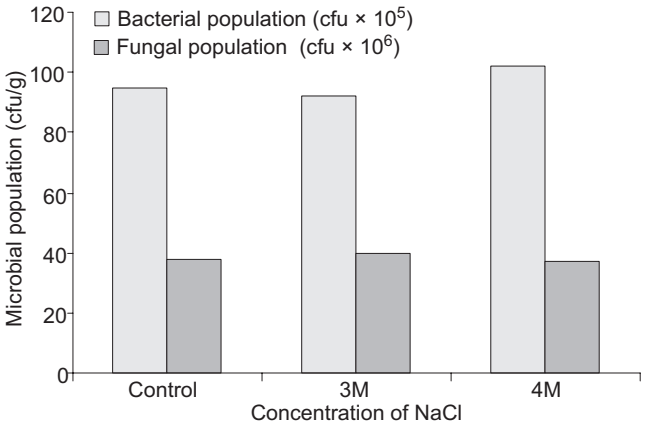

Fig 3 Effect of NaCl on soil microflora

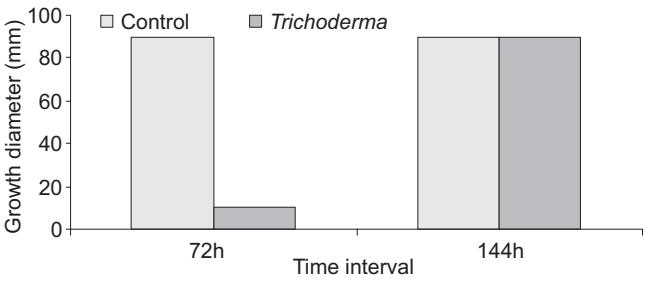

Fig 4 Effect of NaCl on *Trichoderma*

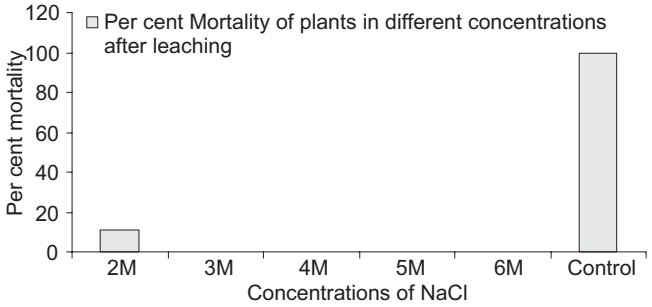

Fig 5 Mortality of the plants after nullifying the differential treatments

Though fungicidal, it is clear from the studies that, this chemical can be used only for disinfesting the pathogen at its source and not for controlling the disease as a soil treatment. Certain eradicative fungicides are toxic to plants but useful for suppressing *Phytophthora* in soil and water and on glass house benches. One such compound used was sodium hypochlorite which releases free chlorine into water which in turn is inhibitory to *Phytophthora* species. Smith (1979) reported that chlorine released from Sodium hypochlorite at 2 mg/litre killed zoospores of *P. cinnamomi*, following an exposure of 1 min. at 18°C. However 100–200 mg of chlorine was required to kill mycelium, probably because *P. cinnamomi* produces thick walled chlamydospores. In our studies it was found that 175 mg/ml was required to kill the fungus in the soil. Grech and Rijkenberg (1992) from their greenhouse studies reported that chlorine at 200–500 mg/ml reduced propagules of *Phytophthora* in soil. But the dosage of chlorine compounds must be kept below the toxicity level to plants.

It is inferred that NaCl can be used for disinfecting the soil but not advisable to apply directly to the plants. This method can be used as a pre planting practice, while rejuvenating a diseased garden or while gap-filling or while raising nursery plants in potting mixture. It is also found that the inhibitory concentration is not harmful to *T. harzianum* or other microflora as shown by the microbial biomass after treatment in comparison with control. No significant difference in population level was noticed in these inhibitory concentrations. This was in fact supported by the studies conducted elsewhere and reported by Elmer (2003) from Connecticut Agricultural Experimental Station, USA. According to Elmer, NaCl is not fungicidal in soil since most soil-borne pathogens grow better in culture as NaCl concentrations are increased to 0.5–1.0%. Moreover, densities of pathogens in soil remain relatively unchanged. Hence, it is assumed that the fungicidal action shown by NaCl against *Phytophthora* is somewhat specific.

Results of the nutrient analysis showed that there was no significant change in nitrogen content or phosphorus content with the maximum inhibitory concentration. However, in case of phosphorus, 6 M showed significantly lower content than in any other concentration when compared to control but this concentration is not required. The phosphorus content in other concentrations are at par with control, indicating that salt treatment at the inhibitory concentration is not affecting the P content of the soil. There is a reduction in potash content which is significantly lower than that in control. This is because K is replaced by Na which is higher in all the concentrations due to addition of NaCl.

Though sodium is not essential in plant growth, some studies have shown that Na can substitute for K when K levels are low. Similarly, plant roots readily absorb chloride, though the amount of chlorine required by the plant is extremely low, high rates of chloride have notably positive effect on soil-plant relations. The salt treatment to reduce *Phytophthora* population is possible before planting, lest they may get wilted. First the soil should be treated with the salts and watered properly for at least 10 days or apply the salt during the premonsoon showers and leave the soil fallow for at least 4–5 rains. The soil is then planted, which will be free from *Phytophthora* population. Continuous washing will remove the salt content from the soil and prevent the plants from phytotoxicity. Thus, the study indicated that the ITK of NaCl application indeed helps in reducing the pathogen population in soil and could be used as soil disinfectant to rid of *P. capsici* in soil. It is also economical and ecofriendly when cost-effectiveness is considered.

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