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Research Article

Control of Soil-borne Pathogens by Soil Fumigation with Paraformaldehyde (Fogidesfarm) as Alternative to Methyl Bromide

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Abstract

Background and Objective: Soil-borne pathogens cause serious and economic losses in vegetable production worldwide. The present study aimed to evaluate the soil fumigation with paraformaldehyde (Fogidesfarm) as alternative to methyl bromide to control soil-borne pathogens in a series of laboratory tests, greenhouse pot experiment and greenhouse trials. **Materials and Methods:** In laboratory tests, soil samples were taken from fumigated and non-fumigated infested soils to prepare soil solutions, 1 mL from each were cultured on PDA media and incubated at $25\pm2^{\circ}C$ for 48 h, the number of colonies were counted. Effect of paraformaldehyde on seed germination and plant seedlings was estimated in greenhouse experiments by direct plantation of tomato, cucumber and pepper seeds and transplanting of seedlings of the same vegetable crops in fumigated and non-fumigated soils. Paraformaldehyde was tested for controlling root knot nematode through comparison of root knot formation on tomato roots in both fumigated and non-fumigated soils after four months of planting in greenhouse pot experiment. The statistical analysis for this experiment was mainly depended on percentages, frequencies, means and standard deviations for observations. **Results:** Laboratory tests indicated decrease of soil pathogens after fumigation from about 4000 CFU g^{-1} soil to about 40 CFU g^{-1} soil. Results indicated that seed germination in fumigated soil exceeded 95%, where about 95% of seedlings succeeded in all vegetable seedlings in fumigated soils compared with 66% in cumber, 72% in pepper and 62% in tomato seedlings succeeded in the non-fumigated soil. Results also indicated formation of nematode knots on tomato roots in the non-fumigated soils, where no nematode knots appeared on tomato roots planted in the fumigated soil. All experiments indicated high ability of paraformaldehyde to control soil pathogens including root knot nematode on tomato, cucumber and pepper plants without any effect on planted seedlings or seed germination of any of the tested vegetable crops. **Conclusion:** Results of this study demonstrated that paraformaldehyde (Fogidesfarm) has the potential to be used as an effective and save alternative to methyl bromide.

Key words: Paraformaldehyde (Fogidesfarm), soil-borne fungi, root knot nematode, soil fumigation, methyl bromide, tomato, cucumber, pepper seeds

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Paraformaldehyde is a polymerization product of formaldehyde with a typical degree of polymerization of 8-100 units with a chemical formula $HO(H_2CO)_n H$, n = 8-100[1]. The basic units of paraformaldehyde (HCHO) molecules dissolve rapidly in water forming methylene hydrate molecules that combine together forming formalin which is polymers with 2-8 molecules with about 37-40% formaldehyde and 60-63% water by weight, where higher polymers containing up to 100 molecules form a white powder, paraformaldehyde[2]. Combustion of paraformaldehyde will depolymerize it into its structural units (formaldehyde). The International Programme on Chemical Safety (IPCS) published that the half-life of formaldehyde in urban air ranges between 35 min in the presence of nitrogen dioxide (NO_2) and 50 min in the absence[3] of NO_2. Howard *et al.*[4] estimated half-life of formaldehyde in the soil of 24-168 h based on aqueous aerobic biodegradation half-lives.

Paraformaldehyde could be used as a fumigant, to control microbes mainly in closed systems or as fungicide, bactericide and nematicide[5,6], in addition to its use as general disinfectant[7] which acts through a physical penetration and diffusion into the innermost layers of cells[8,9]. Paudyal *et al.*[10] tested formaldehyde for controlling root knot nematode in tomato and it was found to delay infection for three months after which small galls started to appear on roots below the ground. Costilow *et al.*[11] used paraformaldehyde pellets as a germicide in tree wounds to control microorganisms and found that residual effects of paraformaldehyde did not exceed 2 ppm, whereas Baraniak *et al.*[12] used paraformaldehyde in tree wounds and reported that formaldehyde residues was 0.74 ppm which might be representative of typical levels of formaldehyde in maple syrup. Hanks[13] used formaldehyde to control narcissus bulb and stem nematode through treatment of narcissus stems and bulbs with formalin (38-40% formaldehyde) for 60 or 15 min at 46 and 48°C, respectively without causing any crop damage. Neshev[14] pointed that formalin is widely used to control fungi and bacteria in both soil and compost, in addition to its ability to control different genera of nematode including *Pratylenchus, Meloidogyne, Rotylenchus* and *Heterodera*.

Formalin was also added to skim milk fed to pigs[15] and as preservative to a wide variety of food products to protect from spoilage[16], in addition to its use in cosmetics industry and baby toilet tissues [17] and as antimicrobial agent in hand cream, make up mouthwashes and others[6,18,19]. Hansch and Leo[20], pointed that formaldehyde was not expected to bioaccumulate due to its bioaccumulation factor (0.19)[21]. The no-observed-effect levels of formaldehyde in animals[20] was 50-75 mg kg^{-1} b. wt. day^{-1}.

After banning of use and production of methyl bromide (the most effective soil fumigant used), farmers started looking for safe alternatives to this fumigant to control soil pathogens that cause severe and economic losses of different vegetable crops all over the world[22]. Several agricultural practices including soil solarization, biotic solarization and combination between both were used but their effect on soil pathogens was limited[23-25]. Soil solarization and other means used were found to weaken soil pathogens more than killing them[26,27]. Characters of paraformaldehyde show that it could be promising compound and may act as safe and effective alternative to methyl bromide.

Therefore, this study aimed to use and applicate paraformaldehyde (Fogidesfarm) product as alternative to methyl bromide in soil fumigation for agricultural soils under greenhouse conditions and in open fields.

MATERIALS AND METHODS

Isolation and identification of soil pathogens: Soil samples and roots of symptomatic plants from previously planted plastic house were used for identification of soil pathogens. Identification was based on morphological features of fungal growth on PDA media and microscopic diagnosis of fungal somatic structures[28].

Fumigation in plastic sacks: Plastic sacks were used in this treatment (to fumigate soil samples *in vitro*) where about 20 kg soil from heavily infested plastic house was placed in each of four sacks. About 10 g of paraformaldehyde (Fogidesfarm) was used to fumigate each of three sacks and one was remained as control. Soil samples were taken from both treated soils and control. Ten grams (dry weight) from each were used to prepare soil solution, diluted and isolated on PDA media, then incubated at 25±2°C for 48 h in the laboratory[29]. Soil-borne fungi were expressed as number of colony forming units per one gram of the soil on PDA media[28,30].

Fumigation in the field: A plastic house with area about 270 m^2 was used for this purpose, the soil was well-plowed twice, smoothed, leveled and prepared for fumigation. Fogidesfarm blocks (paraformaldehyde) were placed in furrows with about 30 cm depth in the prepared soil and with a space about 15 m between each two blocks, burned for 5 min, then the fire was extinguished and polyethylene mulch 100 μ in thickness was used to cover the burned blocks and soil furrows. Treated furrows were left for 24 h, then mulch was removed and beds were prepared for planting. Five beds were prepared in the plastic house each about 60 cm in width with about 1.5 m between each tow beds. Four beds were treated and one bed was left as control. Soil samples were taken from both treated soils and control. Ten grams (dry weight) from each were used and tested as in case (a). Beds were irrigated for 5 h before planting.

Seedlings of tomato, cucumber and pepper were planted seven days after fumigation and visual observation was carried on daily to estimate any disorders of plants. Final estimation was carried on after four weeks of plantation. Parameters were plant length, number of leaves and number of dead plants in each treatment compared to the control.

Test for seed germination: Seeds of cucumber, pepper and tomato were planted directly in treated soil and control. Percentage of seed germination was collected to evaluate effect of paraformaldehyde (Fogidesfarm) on seed germination.

Test for nematode control: Heavily infested soils were collected from different farms in Jordan Valley, three sacks were fumigated with paraformaldehyde (Fogidesfarm) and one was left as control. Four pots from each sack were used for experimental planting and four one as control. All pots were planted with tomato seedlings in April 2016 and monitored up to July 2016. Plants then were removed from pots, roots of these plants were washed and efficiency of the fumigant was evaluated upon appearance of nematode galls on roots of tomato plants.

Statistical analysis: The statistical analysis for this experiment was mainly depended on percentages, frequencies, means and standard deviations for observations.

RESULTS AND DISCUSSION

Soil fungi were isolated from roots of symptomatic plants collected from the previous planting season in the plastic house used for fumigation and found to be: vascular wilt fungus *Fusarium*, damping-off (fungal-like organism) *Pythium*, root rot fungus *Rhizoctonia* and cottony stem rot fungus *Sclerotinia scerotiorum*, in addition to root knot nematode *Meloidogyne* spp.

Fig. 1(a-b): Soil fumigation in (a) Plastic sacks and (b) In the field

Table 1: Average number of isolated fungi in both treated and untreated soils

Treatments	1	2	3	4
	(CFU g^{-1})			
Plastic sacks (treated)	50	50	60	40
Plastic sacks (un-treated)	3350	3450	3500	3200
Field isolation (treated)	40	80	70	60
Field isolation (un-treated)	3750	3800	3650	3500

Soil fumigation with paraformaldehyde (Fogidesfarm) decreased fungal population from about 3800 CFU g^{-1} soil to 40-80 CFU g^{-1} soil (Table 1). In treated soils in both plastic sacks and field experiment (Fig. 1), number of CFU g^{-1} soil ranged between 40 and 80, where in the control of both plastic sacks and field experiment, number of colony forming units per gram soil ranged between 3200 and 3800 CFU g^{-1} as shown in Table 1 and Fig. 2.

Results indicated high efficiency of paraformaldehyde (Fogidesfarm) to control soil pathogens. Paraformaldehyde was also used in sterilizing closed systems in poultry farming against different bacterial and fungal pathogens[14] and to disinfect hatching eggs[31].

Other previous studies pointed that formalin was used as fungicide, bactericide and nematicide and was effective disinfectant against many other microbes[11,29]. Formaldehyde was also used as disinfectant for brooding houses and fodder preservative[32]. Most previous alternatives to methyl bromide were applied as liquids either to be drenched with irrigation water or separately, where the other pesticides used were added as granules in addition to soil solarization[14,33,34], where paraformaldehyde (Fogidesfarm) is applied as fumigant and

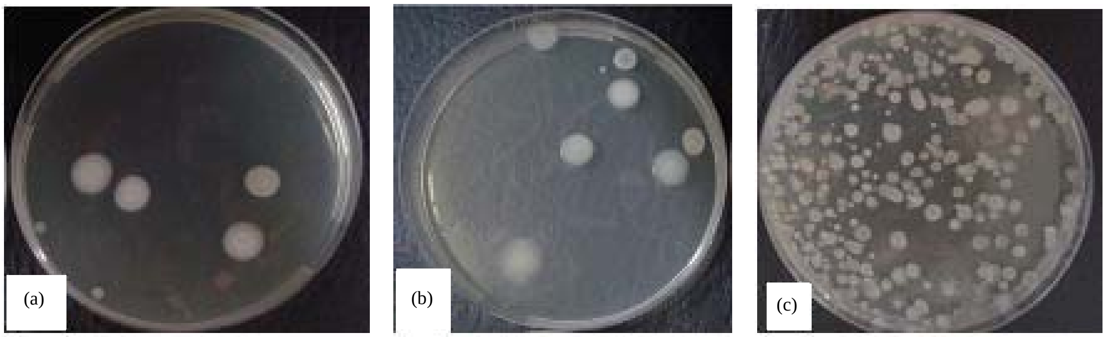

Fig. 2(a-c): Number of colony forming units per gram soil (a, b) In fumigated soils and (c) Control

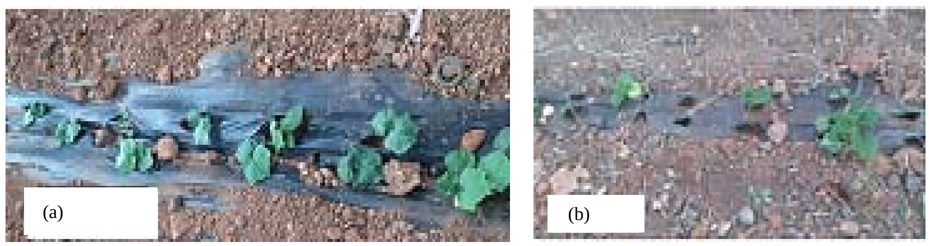

Fig. 3(a-b): Cucumber plants (two weeks after plantation) (a) Treated soil and (b) Control

Table 2: Initial assessment of different parameters of planted vegetable crops two weeks after plantation

Vegetable crops	Parameters	Treatments	Control
Cucumber	Total number of plants	200	50
	Number of dead plants	9	17
	Percentage of succeeded plants (%)	95.5	66
	Average plant length (cm)	48	41
	Average number of leaves/plant	9	7
Pepper	Total number of plants	200	50
	Number of dead plants	11	14
	Percentage of succeeded plants (%)	94.5	72
	Average plant length (cm)	13	9.5
	Average number of leaves/plant	9	9
Tomato	Total number of plants	200	50
	Number of dead plants	10	19
	Percentage of succeeded plants (%)	95	62
	Average plant length (cm)	12	9
	Average number of leaves/plant	5	4

can penetrate through soil particles resulting in controlling soil pathogens more efficiently.

Effect of paraformaldehyde (Fogidesfarm) on plants was studied and evaluated through daily observations of planted seedlings and seed germination, the initial recorded assessment was carried for two weeks after plantation, where cucumber plants formed full expanded new leaves and flowers. The percentage of dead plants in treated beds did not exceed 5% compared with about 34% in the control bed. Many studies before, pointed that formalin was used as an alternative to methyl bromide and had no effect on seeds or plants and considered as nonpathogenic to plants which confirms the results in this study[11].

In pepper seedlings, about 5.5% of seedlings were died in treated beds compared with about 28% in the control bed (Table 2), where in tomato seedlings, about 5% of seedlings were died in treated beds compared with about 38% in the control one as shown in Table 2 and Fig. 3-5. Death of seedlings in non-treated beds was attributed to heavy soil infestation with soil pathogens, which was clear in the soil samples isolated from it and was indicated in Fig. 2. Infection was clear on roots of dead plants in the non-treated soil and

Fig. 4(a-b): Pepper plants (two weeks after plantation) (a) Treated soil and (b) Control

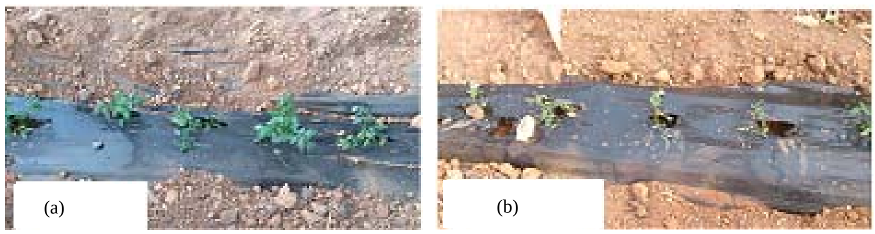

Fig. 5(a-b): Tomato plants (two weeks after plantation) (a) Treated soil and (b) Control

Fig. 6: Seed germination in fumigated soil with paraformaldehyde

upon isolation on PDA media several root rot fungi were isolated, mainly *Fusarium solani* and *Pythium ultimum*.

Results of plant length and average number of leaves in Table 2 indicate clearly that paraformaldehyde (Fogidesfarm) had no phytopathogenic residual effects on the different vegetable plants used in this experiment which is in agreement with previous studies[32].

Figure 3-5 show clear effects of paraformaldehyde (Fogidesfarm) on soil pathogens through growth of plants in fumigated soil compared with non-fumigated (control). All parameters including percentage of succeeded plants, plant length, color of plants and number of leaves/plant indicated the beneficial effects of soil fumigation with Fogidesfarm. On the other hand, growth of weeds in fumigated soil indicated that paraformaldehyde (Fogidesfarm) has no phytopathogenic effect on plant seeds and weeds even when directly subjected to the fumigant.

Seed germination in fumigated soil was tested for cucumber seeds to insure that fumigant (Fogidesfarm) has no effect on seeds and results indicated that germination of seed in fumigated soil exceeded 95% which is similar to germination in peat media used generally for this purpose as shown in Fig. 6.

Many previous studies pointed that paraformaldehyde had no phytopathogenic residual effects on plants or seeds and could be applied directly to control plant diseases or pests[34-36]. Final assessment was carried on after 28 days from plantation to insure the efficiency of paraformaldehyde (Fogidesfarm) in controlling soil-borne plant pathogens and its effect on growth and development on plants in treated soil beds compared with control. In all vegetable plants planted in the experiment, growth of plants in treated soil beds was normal and plant length in all treatments exceeded that in the control as shown in Table 3 and Fig. 7-9.

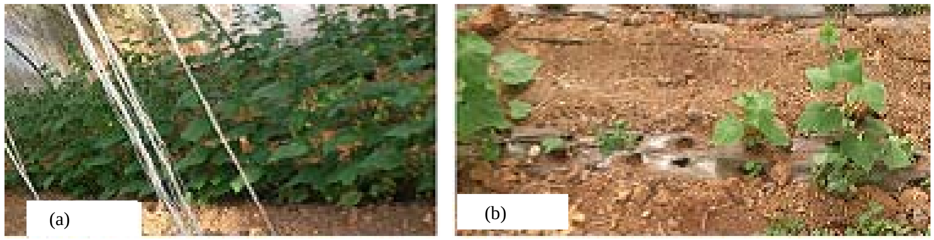

Fig. 7(a-b): Cucumber plants (four weeks after plantation) (a) Treated and (b) Control

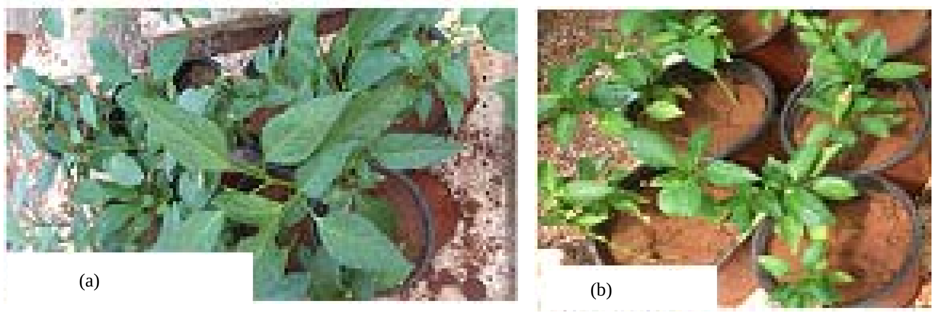

Fig. 8(a-b): Pepper plants (four weeks after plantation) (a) Treated soil and (b) Control

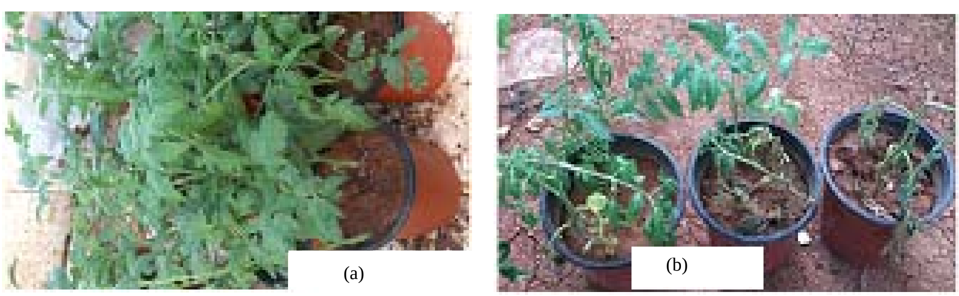

Fig. 9(a-b): Tomato plants (four weeks after plantation) (a) Treated soil and (b) Control

Results of Table 3 show that succeeded vegetable plants in fumigated beds continued their growth and development without being subjected to any pathogenic organisms compared with those in the non-fumigated bed (control). Figure 7-9 show the growth of planted vegetable plants after 28 days of plantation. This indicated the efficiency of paraformaldehyde (Fogidesfarm) in controlling soil pathogens and preventing plants from soil-borne diseases. Neshev[14] pointed that formalin when used as fumigant, penetrates in to the soil up to 20-30 cm depth controlling soil fungi including the genera *Pythium, Phytophthora, Rhizoctonia, Fusarium, Verticillium* and many others[11].

Figure 7-9 clearly indicate positive effects of fumigant on the growth of plants in treated beds/pots compared with the control one.

Test for nematode control indicated that all tomato plants in fumigated soil with Fogidesfarm did not show any galls on their roots caused by the root knot nematode *Meloidogyne* spp. after four months from plantation, where in the control pots galls appeared clearly on tomato plat roots after the same period of time. This was approved before by Neshev[14] who pointed that formaldehyde control several genera of nematodes including the root knot nematode *Meloidogyne*[11]. Previous studies pointed that when formaldehyde was used to control root knot nematode, it delayed infection for about

Table 3: Final assessment of different parameters of planted vegetable crops four weeks after plantation

Vegetable crops	Parameters	Treatments	Control
	Total number of plants	200	50
	Number of dead plants	11	20
Cucumber	Percentage of succeeded plants (%)	95.5	60
	Average plant length (cm)	148	141
	Average number of fruits/plant	9	7
	Total number of plants	200	50
Pepper	Number of dead plants	13	15
	Percentage of succeeded plants (%)	93.5	70
	Average plant length (cm)	38	29
	Average number of leaves/plant	19	15
	Total number of plants	200	50
	Number of dead plants	11	20
Tomato	Percentage of succeeded plants (%)	94.5	60
	Average plant length (cm)	47	38
	Average number of leaves/plant	10	8

three months which means that the source of infection was from the untreated soil beneath the root zone and this is what was approved in the experiment[7].

CONCLUSION

The results of this study demonstrated that paraformaldehyde (Fogidesfarm) has the potential to be used as an effective alternative to methyl bromide as soil fumigant to control soil borne pathogens including root knot nematode and soil insects without any observed adverse effects on plants.

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