# Reduction of Soil-Borne Plant Pathogens Using Lime and Ammonia Evolved from Broiler Litter

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**Key Words:** Bell-pepper, Greenhouse, Lime, Soil pH, Solarization

## Abstract

*In laboratory and micro-plots simulations and in a commercial greenhouse, soil ammonia ($NH_3$) and pH were manipulated as means to control soil-borne fungal pathogens and nematodes. Soil ammonification capacity was increased by applying low C/N ratio broiler litter at 1–8% (w/w). Soil pH was increased using lime at 0.5–1% (w/w). This reduced fungi (Fusarium oxysporum f. sp. dianthi and Sclerotium rolfsii) and root-knot nematode (Meloidogyne javanica) in lab tests below detection. In a commercial greenhouse, broiler litter (25 Mg $ha^{-1}$) and lime (12.5 Mg $ha^{-1}$) addition to soil in combination with solarization significantly reduced M. javanica induced root galling of tomato test plants from 47% in the control plots (solarization only) to 7% in treated plots. Root galling index of pepper plants, measured 178 days after planting in the treated and control plots, were 0.8 and 1.5, respectively, which was statistically significantly different. However, the numbers of nematode juveniles in the root zone soil counted 83 and 127 days after pepper planting were not significantly different between treatments. Pepper fruit yield was not different between treatments. Soil disinfection and curing was completed within one month, and by the time of bell-pepper planting the pH and ammonia values were normal.*

## Introduction

The problems of maintaining adequate soil quality and fertility, and of protecting crops against soil-borne diseases are major obstacles to profitable organic production of greenhouse vegetables. In the desert Arava Valley, Israel, bell-pepper and tomatoes are grown from September to June and the areas are left barren during the extremely hot summer. This is conducive to establishment of soil-borne pathogens (Cook, 1981).

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Soil solarization followed by incorporation of green manure or manure compost is a primary means to prevent the spread of disease, but success is limited. Ammonia ($NH_3$) released from decomposing manure is used to control soil-borne diseases (Eno et al., 1955; Smiley et al., 1970; Tsao & Oster, 1981; Rodríguez-Kábana et al., 1982; Oka et al., 1993; Lazarovits et al., 2000; Lazarovits, 2001; Oka et al., 2006). Despite the known nematicidal and antifungal activity of ammonia, its use as a soil disinfectant is hindered by the heavy doses needed for effective pest control and consequent

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phytotoxicity (Rodríguez-Kábana et al., 1987; Stirling, 1991). Raising soil pH alters the benign ammonium ($NH_4^+$) to the biocide ammonia according to the Henderson-Hasselbalch (H-H) equation (log $[NH_{3(g)} / NH_4{}^+{}_{(aq)}] = pH - 9.3$). Combined application of ammonium fertilizer (at 60–250 mg N/kg) and lime (as alkaline-stabilized biosolids at 75 Mg $ha^{-1}$) to a neutral sandy soil in a field micro-plot trial reduced *Fusarium oxysporum* f. sp. *dianthi*, *Verticillium dahliae* below detection limit (Ben-Yephet et al., 2005). With adequate application rates, soil pH quickly decreased to normal, allowing ammonium to nitrify (Focht and Verstraete, 1977). In the present study, we examined the nematicidal and fungicidal activity of combinations of lime and broiler litter (BL) applied to neutral and basic sandy soils.

## Methodology

### Laboratory tests

**Fungi:** A sandy-loam soil naturally infested with *F. oxysporum* f. sp. *dianthi* was used. Soil samples, each of 50 g, were packed in 50-ml cups. Broiler litter was added at 0–80 g $kg^{-1}$ on dry weight basis, and mixtures were moistened to water holding capacity and incubated at 27°C for up to 20 days. Cement kiln dust (CKD) at 1–10 g $kg^{-1}$ was mixed with the soil 3–7 days after onset. Five grams soil samples were tested for *F. o. dianthi* counting (Ben-Yephet et al., 1996; 2005), pH and total ammonium. Sclerotia of *Sclerotium rolfsii* that were placed inside the mixtures were recovered and plated on selective growth media (PDAS). The experiment was repeated each time in triplicates.

**Nematodes:** The BL was pulverized and mixed with 10 g dune sand in 25-mL glass vials at concentrations of 0, 1, and 2 g $kg^{-1}$. Approximately 300 *M. javanica* J2s were administered in 1.3 mL of water. The vials were capped and incubated at 27°C. Three days later, CKD at final concentrations of 0, 1 and 2 g $kg^{-1}$ was mixed with the sand, and the incubation was continued for 4 days more. Then, mobile J2s were recovered from the soil on a 60-μm sieve and counted.

**Nematodes, tomato assay**: One kg dry sand was amended with BL at 0, 1, 2 and 4 g $kg^{-1}$ ratios and with 2,500 *M. javanica* eggs + J2 (at 2:3 ratio; in 5 ml water). After 4 days incubation at 27°C CKD was (factorially) mixed well with the soil at 0, 1 and 2 g $kg^{-1}$, and the incubation continued for 4 days. Then, one month old tomato (cv. Daniela) seedlings were planted in the pots. The seedlings were uprooted 6 weeks later, and the fresh shoot weights and number of nematode eggs on the roots were recorded. The galling index (GI) of the roots was assessed on a 0 to 5 rating system: 0, no infection; 1, 1–20; 2, 21–40; 3, 41–60; 4, 61–80; and 5, 81–100% of roots galled.

### Greenhouse experiment

An organic farming greenhouse sandy calcareous soil infested with *M. incognita* was amended with commercial cattle manure compost at 10 liter $m^{-2}$. The plot was divided into six units; 5 m × 8 m each, each subdivided in to 5 beds, 1m × 8 m each. The initial nematode population counted by Baermann funnel method was 0.7 J2 per 50 g soil in the 20–30 cm depth. Broilers litter and slaked lime were applied (28 July, 2004) on the bed surface at 4.2 kg $m^{-2}$ (≈ 10 g $kg^{-1}$) and 2.5 kg $m^{-2}$ (≈ 0.6 g $kg^{-1}$), respectively, which was intended to transiently increase soil pH from 8.1 to 10. The additives were mixed into the soil to a depth of 30 cm. Two drip irrigation laterals were placed on each planting bed, and each experimental unit was separately covered with a polyethylene sheet. The plots were equally irrigated at 15 liter $m^{-2}$, and the irrigation was repeated 8 days later. The polyethylene cover was removed on the 12th day. After uncovering, the soil was allowed to dry out for 8 days, and the ammonia to volatilize. Irrigation with 15 liter $m^{-2}$ was given on the 20th and 25th day.

Bell pepper (*Capsicum annuum* cv. Dimano, Makhteshim, Israel) seedlings were planted on the 29th day. Planting was in two rows in each planting bed, at 200 plants per experimental unit. Nematode-susceptible tomato plants (cv. Daniela) were planted between the pepper plants (10 plants per experimental unit) 40 days after treatment and uprooted 35 days later. Tomato plants infected with nematodes were counted. During the growth, number of the nematode juveniles per 50 g soil in the root zone (about 10 cm from the stem, and at 10 cm depth) was counted 83 and 127 days after pepper planting. Root galling index (as above) of the fifty plants was recorded 178 days after planting. The plants received guano and feather meal during the growth period as common in the region**.** On each harvest event (from 15 Dec. 2004 until 11 April 2005), pepper fruits from 10 plants per bed were sampled.

## Results and Discussion

**Fungi reduction in the lab:** During the first 10 days after application, ammonium concentrations in the BL-amended soil ranged from 230 to 590 mg $kg^{-1}$ (Table 1). Soil pH either did not change (at 8.4–8.7) during this period or it somewhat decreased. The addition and incubation increased *F. o.* f. sp. *dianthi* counts by up to 10 times. Mixing CKD with the soil at 10 g $kg^{-1}$ seven days after incubation onset, raised soil pH to 9.6–11; the increase was inversely related to litter loading rate. The addition of CKD alone reduced *F. o.* counts in the soil almost below detection limit, and in combination with the manure it was reduced below it (Table 1). *Sclerotium rolfsii* was more sensitive to either high pH or $NH_3$ (Table 1). Peak $NH_3$ concentrations in the solution phase of the (manure+lime)-treated soils were 500–1065 mg $l^{-1}$ (calculated using the H-H equation).

**Nematodes, incubation study:** CKD (1–2 g $kg^{-1}$) and/or BL (1 or 2 g $kg^{-1}$) addition to the sand reduced the numbers of J2s recovered (Table 2). CKD alone at 1 and 2 g $kg^{-1}$ (pH 10 and 10.8) reduced J2 nematode respective recoveries by approx. 17% and 65% of those in the untreated controls. The combinations of BL at 1.0 or 2.0 g $kg^{-1}$ and CKD at 1.0 g $kg^{-1}$ increased the pH to 9.9 and reduced J2s recovery below detection levels.

**Tomato assay**: The tomato fresh shoot weight (FSW) increased with increasing BL application rate (1–4 g $kg^{-1}$; Table 3). At each litter application rate, combining with CKD reduced or removed the weight increase. Increased applications of either CKD or BL alone reduced the GI of tomato roots but the combination of the two was more effective. The nematode infestation of the roots (number of eggs per root) was reduced only by combinations of higher BL and CKD doses.

**Commercial greenhouse experiment**: Application of lime increased the pH of the BL and compost treated soil from 7.5 to 11 (Fig. 1A). The pH was ≥ 10 for about a week, and it decreased to less than 9 by the 12th day after treatment, and then to 8 by the 29th day. The mean ammonium concentrations in the soil persisted at > 200 mg N $kg^{-1}$ from the 5th day through the 29th day after treatment (Fig. 1B). Estimated concentrations of gaseous ammonia exceeded 25 mg N $kg^{-1}$ for some 20 days, peaking at >60 mg N $kg^{-1}$ (Fig. 1C). Initial nitrate concentration was high (> 300 mg N $kg^{-1}$) in all plots (Fig. 1D). The concentrations reached a minimum (50 mg N $kg^{-1}$) on the 12th day and then increased to 600 mg N $kg^{-1}$ or more. The increase in the control plots was steeper than in the treated plots, yet to a somewhat lower value, and it decreased later on. Nitrite ($NO_2^-$) concentrations in the BL treated plots were significant throughout most all this period, at 5–50 mg $NO_2^-$-N $kg^{-1}$ (not shown).

The efficacy of the treatment is indicated by the GI of the tomato test plants. Whereas 47% of the plants in the control plots were infected with *M. javanica*, only 7% of those in the treated plots were infected. However, 83 and 127 days after pepper planting, the numbers of nematode juveniles in the soil near the bell-pepper roots were not significantly different between the manure-treated and untreated plots. Similarly, the bell-pepper fruit yields were not significantly different between the treatments. This was probably because of upward migration of nematode larvae later in the season and from side contamination.

It should be emphasized that (i) target ammonia concentrations and pH values were obtained at both soil disinfection and soil curing stages, and (ii) damage to tomato test plants from the root-knot nematode *M. javanica* was drastically reduced. This was despite that the polyethylene cover was removed already on the 12th day due to season constraints. In the Arava Valley, 30–60 days soil solarization is used in attempt to control soil-borne diseases. Longer solarization combined with the ammonia-pH treatment could perhaps improve nematode control. Higher soil temperatures at solarization and higher ammonia vapor pressure are also expected to increase ammonia diffusion to deeper soils, improve treatment efficacy, and make it possible to reduce amendment rates (by reducing the pKa of the H-H equation).

Two additional issues are worthy of mentioning: (i) the treatment affects beneficial soil microflora too (which is worthy of assessment), and thus should be applied with compassion. (ii) No single treatment (this or another) should be expected or attempted to alleviate a chronic soil disease.

**Compatibility with organic regulations**

Reducing soil-borne plant pathogens using ammonia evolved from manure is an old concept in organic agriculture, often an unsuccessful one. Arid and semi-arid basic, light-textured soils are more conducive to this procedure. The compliance with organic regulations of the treatment components (lime and manure) and concept should be evaluated. **Lime**: NOP rules restrict the use of hydrated lime, $Ca(OH)_2$, for controlling crop pests, weeds and diseases (CP) (Rule reference 205.601(i)(3). The rules prohibit the use of lime as a 'crop fertilizer and soil amendment' (CF) (Rule 205.105(a)) (OMRI, 2004). IFOAM regulations permit application of quicklime for crop protection and growth regulation (OMRI, 2004; p. 101). Wood ash or basic slag which is approved as a fertilizer and soil conditioner by European and international agencies (EC reg. no 2092/91 + Council proposal 8697/98, Codex Alimentarius 99/22 and IFOAM Standards) could yet be used. **Manure/compost**: NOP rules allow application of raw uncomposted manure to edible crops if 90-120 days elapse between application and harvesting. IFOAM and EC regulations do not restrict the application of 'organic' raw manure, but the application of manure from 'factory farming' is either not allowed or allowed after composting. In this context, broiler breeding is not considered 'factory farming'. **General concept:** Arid and semiarid soils do not accumulate much organic matter despite heavy compost applications under organic greenhouse regime, certainly in the open field. More attention has to be paid to soil quality, fertility and biology under this regime, especially as crop rotation is often avoided due to lack of crop selection. Hence, soil pest might persist and adequate means (and perhaps integrated means) should be made available to growers.

## Conclusions

We demonstrated that ammonia generated by ammonification of broiler litter with relatively high N content and low C/N ratio, in combination with a transient increase of soil pH, can effectively control soil-borne diseases, the manure also being source of available plant nutrients. Attention should be given to organic-approved source materials for ammonia and hydroxyls. In addition, the pest control should be well tuned with respect to target organisms and soil quality.

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Table 1: Effect of broiler litter and CKD on soil $NH_4$, $NH_3$ and pH and on viability of test fungi

| Treatment | Max. $NH_4$ (mg $kg^{-1}$) | Max pH | Estimated Max $NH_3$ (mg $l^{-1}$) | *F.o.d.* (cfu $g^{-1}$) | *F.o.d.* (% reduction) | *S. rolfsii* (% reduction) |
|---|---|---|---|---|---|---|
| Soil only | 2 | 8.7 | 2 | 1,200 | - | - |
| No addition | 6 | 8.7 | 4 | 1,222 | 0 | 0 |
| 10 g $kg^{-1}$ BL | 236 | 8.7 | 156 | 14,000 | Increase | 0 |
| 20 g $kg^{-1}$ BL | 482 | 8.8 | 401 | 11,722 | Increase | 37 |
| 40 g $kg^{-1}$ BL | 588 | 8.5 | 277 | 3,111 | Increase | 20 |
| 80 g $kg^{-1}$ BL | 568 | 8.4 | 192 | 1,611 | Increase | 52.5 |
| 10 g $kg^{-1}$ CKD | 2 | 11.3 | 11 | 100 | 99 | 8.4 |
| 10-to-80 g $kg^{-1}$ BL +10 g $kg^{-1}$ CKD | 104 | 11.0–9.6 | 503–1065 | 0 | 100 | 100 |

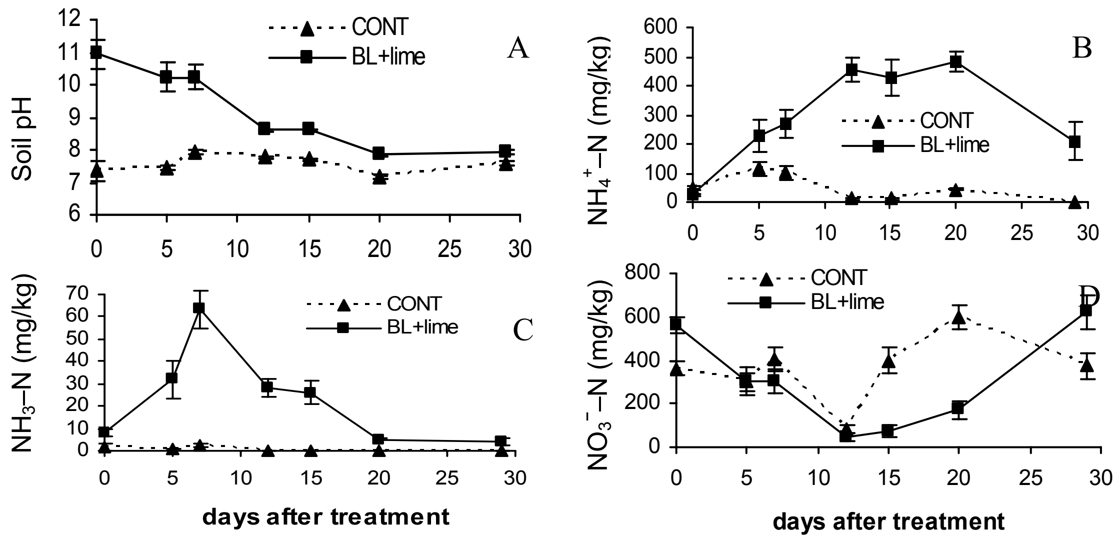
**Table 2:** Number of *Meloidogyne javanica* second-stage juveniles (J2) recovered from soils, and soil pH following treatment with broiler litter, with or without cement kiln dust (CKD).

| | | Broiler litter (g $kg^{-1}$) | | |
|---|---|---|---|---|
| | | 0 | 1.0 | 2.0 |
| Experiment A | CKD (g $kg^{-1}$) | | J2 No. | |
| | 0 | 113.2 a | 31.3 a | 0 |
| | 1.0 | 88.5 a | 0 b | 0 |
| | 2.0 | 40.0 b | 0 b | 0 |
| Experiment B | CKD (g $kg^{-1}$) | | J2 No. | |
| | 0 | 102.9 a | 28.0 a | 0 |
| | 1.0 | 92.8 a | 0 b | 0 |
| | 2.0 | 34.6 b | 0 b | 0 |
| | CKD (g $kg^{-1}$) | | Soil pH | |
| | 0 | 8.5 ± 0.1 | 8.4 ± 0.1 | 8.6 ± 0.2 |
| | 1.0 | 10.0 ± 0.3 | 9.9 ± 0.1 | 9.9 |
| | 2.0 | 10.8 ± 0.3 | 10.7 ± 0.1 | 10.3 ± 0.2 |

**Table 3:** Tomato pot assay for the root-knot nematode *Meloidogyne javanica* (significance tested by Tukey test at $P$ = 0.05; *after log 10 ($x$+1) transformation)

| | Materal applied and rate of application (g kg$^{-1}$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Unamended Control | CKD 0.1 | CKD 0.2 | BL 0.1 | BL 0.2 | BL 0.4 | BL 0.1 CKD 0.1 | BL 0.2 CKD 0.1 | BL 0.4 CKD 0.1 | BL 0.1 CKD 0.2 | BL 0.2 CKD 0.2 | BL 0.4 CKD 0.2 |
| FSW (g) | 11 f | 10.1 f | 10.7 f | 18.7 cde | 24.1 bc | 32.1 a | 15.8 def | 20.6 cd | 29.8 ab | 14.3 ef | 15.8 def | 18.4 cde |
| GI | 3.4 a | 3.4 a | 1.5 bc | 3.1 a | 3.9 a | 2.1 b | 1.7 b | 0.6 cd | 0 d | 0.7 cd | 0.1d | 0 d |
| eggs/plant (×10$^{3}$) | 60 a | 38 a | 37 a | 58 a | 79 a | 45 a | 34 a | 20 a | 0 c | 1.4 b | 0 c | 0 c |

**Fig. 1:** Soil pH (A) and concentrations of ammonium (B), ammonia (C), and nitrate (D) of CONT plots treated with cattle manure compost at 10 liter m$^{-2}$ and plots treated in addition with broilers litter at 4.2 kg m$^{-2}$ plus slaked lime at 2.5 kg m$^{-2}$ (BL+lime). Values are M ± SD of five replicates.


A
Soil pH
CONT
BL+lime
B
$NH_4^+$–N (mg/kg)
C
$NH_3$–N (mg/kg)
D
$NO_3^-$–N (mg/kg)
days after treatment