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Efficacy of Bio-Fumigation and Soil Solarization on Soil-Borne Onion Pathogens

EFFICACY OF BIO-FUMIGATION AND SOIL SOLARIZATION ON SOIL-BORNE ONION PATHOGENS

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SUMMARY
Bio-fumigation is known to manage many soil-borne pathogens of crops but less is known in onion. Studies aimed at evaluating efficacy of soil solarization, bio-fumigation and their combinations on onion soil-borne pathogens within the context of existing growers' cropping systems were conducted in a replicated split-split plot completely randomized block design in field and in greenhouse experiments. Treatments included; mustard vs no mustard as main plot treatment; canola meal cake, chicken manure and control as sub-plot; and plastic mulch vs no mulch as sub-sub-plot. Mustard grown until flowering after sweet corn harvest was incorporated in the soil along with chicken manure and canola meal cake. Temperatures were measured at 15 cm depth of the soil; the soil was covered by 4 mm thick transparent plastic sheet in designated plots and was made air tight on all sides. Onion was grown in the following season, and assessed for incidence of Fusarium basal rot, Pink Root and plant parasitic nematodes. The higher temperature beneath the plastic mulch was observed as compared to non-mulched soil which was enhanced by the soil amendments by mustard meal cake and chicken manure. Mustard, canola meal cake, chicken manure, and their combination did not affect Fusarium basal rot incidence. Only chicken manure reduced the Pink Root incidence. The combination of mustard, chicken manure and soil solarization was more effective in reducing disease and nematode incidence than single application of each. The combination significantly reduced *Pratylenchus* populations even without soil solarization, and only chicken manure with soil solarization reduced *Meloidogyne* populations.

Key Words: soil solarization, mustard, soil-borne fungal and nematode pathogen.

INTRODUCTION

Colorado is a major onion producer in the USA, with approximately 3400 hectare in 2009 with an average yield of 337-345 metric tons/hectare annually. However, onion production in the state has declined since 1997, in part because of widespread threats such as bacterial and viral disorders, in addition to soil-borne issues, fungal pathogens, phytoparasitic nematodes, and competition from urban expansion. Of these, soil-borne diseases caused by fungi, nematodes and bacteria in onion seriously limit production. Among fungal pathogens, *Fusarium oxysporum* f. sp. c*epae* causing basal rot; *Phoma terrestris c*ausing Pink Root disease, bacteria such as *Pecto bacteria* causing storage rot, and plant parasitic nematodes (PPN) such as *Meloidogyne, Pratylenchus,* and *Ditylenchus dipsaci* have significant impact on onion production.

Fusarium basal rot and Pink Root diseases, commonly observed in Colorado onion fields (Swift *et al*., 2002), are favored by moderate to high soil temperature, frequent cropping to onion, soil compaction, poor drainage, cultivation wounds and low soil fertility. Plant parasitic nematodes reduce yield and predispose plants to infection by other soil-borne pathogens without exhibiting any aerial symptoms; these further complicate management decisions. Of 11 nematode genera reported from Colorado onion fields, some genera with higher frequencies and densities (e.g. *Pratylenchus, Meloidogyne)* were considered important for onion production in Colorado (Pokharel *et al*., 2009). Soil-borne diseases have pronounced impact on onion yields in fields planted for more than 3 years in an onion based crop rotation. Plant survival, bulb size and quality can be reduced and thereby affect crop productivity (up to 60 percent yield loss) and profitability.

Past research results have shown that *Brassica* spp. as green manure and or seed meal cake (Brown and Morra, 2005), and/or chicken manure (Tenuta and Lazarovits, 1999) are effective in reducing soil-borne pathogens through the release of toxic and volatile chemical gases such as isothiocyanate (from *Brassica)* and ammonium (from chicken manure) during the decomposition process with a large amount of such gases escaping into the environment. At the same time, a plastic mulch will enhance the decomposition process if sealed properly with adequate moisture level, especially when the temperature is high, minimizing the escape of such gases into the environment following incorporation of these materials, and raising the soil temperature to levels needed to kill soil-borne pathogen propagules (Katan *et al*., 1991). However, the efficacy of soil solarization alone will be high if it is done when the temperature is high enough to kill soil-borne pathogens, and the soil has proper tilth and appropriate soil moisture levels throughout soil solarization period. The best time for soil solarization in western Colorado falls in the middle of the crop growing season, and growers are not willing to skip a crop only for the treatment application such as soil solarization or growing mustard.

Limited information is available on the efficacy of integrated use of *Brassica*, chicken manure, and soil solarization on soil-borne pathogens of onion. Thus, this experiment was designed to test the efficacy of combined use of mustard, chicken manure, and soil solarization on soil-borne pathogens of onions and to fit these bio-fumigation practices with the existing crop rotation practices used by western Colorado onion growers.
It was hypothesized that incorporating mustard, canola meal cake, and chicken manure into the soil and covering them with plastic mulch will enhance the release of volatile gas through

heating and minimize the loss of volatile gases into the environment. Plastic mulch will augment the efficacy of bio-fumigation and/or soil solarization to manage the soil-borne diseases in onion, and these treatments will be effective even when applied between the two crops typical of growers' existing seasonal cropping pattern.

MATERIALS AND METHODS

A grower's field infected with fungal pathogens such as Fusarium basal rot and Pink Root, and nematodes such as *Meloidogyne, Pratylenchus, and Ditylenchus* in Delta County, CO was selected for this study, and the grower served as the cooperator. These pathogens were confirmed by pre-treatment sampling in the field. Fungal pathogens were isolated and identified, and nematodes were extracted, identified and enumerated. The assessment was done by 10 random samplings from the entire field.

Field experiment

After sweet corn harvest in July 2008, the field was irrigated and prepared by disking. In this field, an experiment was laid out in a split-split plot randomized block design with mustard and no mustard as main factor treatments; canola meal cake, chicken manure and control as sub-factor treatments and plastic and no plastic mulch as sub-sub-factor treatments. The experiment was replicated three times, and one experimental unit (one plot) consisted of 15.3 m x 3.0 m area. Yellow mustard (*Brassica juncea* L., variety Pacific Gold) seeds, obtained from Farmers' Cooperative of Genesee, ID, were broadcasted (6.0 kg/ha) in plots designated for mustard.

Five days after an August 2008 irrigation, all soil incorporation treatments (flowering mustard plants, canola meal cake at 5 tons/ha, and chicken manure at 5 tons/ha) were incorporated into their respective plots by disking (18"). Programmed temperature sensors using Thermochron iButton DS 1922L by Embedded Data System inserted (one sensor in each plot) in the soil at 15 cm depth to monitor temperatures. The sensors were programmed to monitor temperatures four times each day for the entire monitoring period. Due to pre-disking irrigation, big clods formed that were hard to break. Four mm thick clear plastic sheets were laid down in respective plots one day following disking, and the plastic was sealed air tight by putting soil on all sides and trampled by foot. The plastic was removed at the end of October, the soil sensors collected, and temperature data downloaded. Onion was planted in April of 2009 with all recommended cultural practices.

From the main plot of 15 m x 3 m, a sub-plot of 0.6 m x 0.6 m was randomly marked in each plot. Different yield parameters (total yield per plot and number of bulbs, were studied from these sub-plots. Number of basal rot infected bulbs, and pink root infected roots were counted in each plot/sub-plot at pre-harvest or at harvest.

Incidence of Pink Root

Twenty-five onion bulbs, five bulbs each from five different locations per plot, were collected two weeks before onion harvesting. Special care was taken to obtain intact root systems while removing the sample. A shovel did not help to get more intact roots than just pulling onion bulbs due to soil structure. Thus plants were carefully pulled by hand. The roots after washing were evaluated for Pink Root severity with a rating based on visual observation using a modified scale of 1 to 10; where 1 = 10%; 2 = 11-20%; 3 = 21-30%; 4 = 31-40%; 5 = 41-50%; 6 = 51-60%; 7 = 61-70%; 8 = 71-80%; 9 = 81-90% and 10 = 91-100% roots infected with Pink Rot.

Incidence of Fusarium basal rot

At harvest, the bulbs infected with Fusarium basal rot were counted in each sub-plot. However, two weeks before harvesting, Fusarium basal rot infected bulbs were counted for those plants which were pulled for Pink Root severity ratings in the field.

Yield data recording

Onion bulbs were harvested by a mechanical harvester. Onion bulbs in each plot were graded one week after harvesting and curing in the field into medium and jumbo sized grades using an onion grader in each plot. The bulb numbers in each category were counted and yield recorded. After removing the infected onion bulbs, 40 random bulbs were collected in a bag from each plot for storage quality evaluation.

Soil nutrient and nematode analysis

About 1200 cc soils were collected from each plot (15 m x 3 m) from 5 different places (four corners and one in the center) at 15 cm depths just after onion harvest. The soils were brought to the laboratory, mixed well, clods broken and rocks removed. From each plot, a 100 cc soil aliquot sample was processed for nematode extraction, and a 100 cc aliquot was air dried and sent for plant nutrient analysis by Ward Laboratory, Kearney, Nebraska.

Storage quality study

The forty onion bulbs were randomly collected from each plot and used for storage quality evaluation. They were cured in shade for about a month and kept for storage rot study in cool storage (basement of a building) where the winter temperatures were similar to cold storage. This study started in November 2009 until March 2010. In early March the bulbs were assessed based on sprouting, bulb rot, and fitness for human consumption based on visual observation.

Greenhouse experiment

Two greenhouse experiments were conducted following the same timing as the field experiment. Plants were uprooted at maturity and all were evaluated for Fusarium basal rot, Pink Root, and nematode infection as described below. *Fusarium oxysporum* f. sp. c*epae* and *Phoma terrestris* were identified from onion root and bulb samples on isolation from pot experiments.

Experiment 1. The soil samples (about 1200 cc) collected from the field in 2008 immediately after removing the plastic in the field, were taken to WCRC-Orchard Mesa for nematode study, soil nutrient analysis and a greenhouse study. The soil samples left over from nematode and plant nutrient analysis from each plot were put in three clay pots of 250 cc capacity and kept in a greenhouse until the next onion growing season. In 2009 onion growing season, two onion cv. Tioga seeds were planted in each pot and maintained inside the greenhouse until maturity.

Experiment 2. Again the soil was collected from the control plots of the experiment in 2009 after onion harvest to conduct similar greenhouse study at Western Colorado Research Center (WCRC), Orchard Mesa facility. The soil placed in each 500 cc capacity clay pots was subjected to the similar treatment application as in the field in 2009. The pots were laid out in randomized block design with five replications in the greenhouse and onion cv. Tioga was grown in the 2010 growing season inside the greenhouse. At harvest all plants were uprooted, and bulbs and roots were washed. Bulbs were observed for Fusarium basal rot symptoms, and roots for Pink Root disease and nematodes. The same amount of soil sample from each plot was processed for nematode extraction where plant parasitic nematode (PPN) genera studies and their numbers and free living nematode numbers were counted. The temperature was monitored only from a few treatments to compare the field observation because of reduced availability of temperature sensors since many failed during the field experiment.

Extraction of nematodes from soil

Field and greenhouse soil samples were mixed well in the laboratory, clods broken-up and a 100 cc soil sample taken. Milk filter papers were placed on top of stainless steel wire screen and covered with two layers of facial quality tissue paper. The 100 cc soil sub-samples were then spread uniformly over the tissue paper, and the screen, filters, tissue paper, and soil samples were placed in an approx. 18 cm diameter pie pan. Sufficient water was added to ensure the submergence of the soil. Each was then covered on top by another aluminum foil pie pan to minimize evaporation. After 72 hours, the tissue paper, milk filter, and soil were discarded. The remaining nematode suspension from the pie-pan (clear water with nematodes) was collected in a beaker and put in a cold room (maintained at 4^oC) until the nematodes were counted within a week. The nematode suspension was reduced to 250 cc, a 20 cc aliquot sample taken, and the nematode genera identified and counted under an inverted compound microscope with 10x ocular and 4x, 10x, and 20x objectives.

Statistical analysis

Nematode data were transformed to Logx for normality for analysis and analyzed using Proc GLM in SAS software. The means were compared using LSD (P=0.05), and are presented as original numbers in the tables.

RESULTS

Soil temperatures

In the field, the higher and lower temperature ranges varied from $17.0\text{-}28.3^oC$ and $6.9\text{-}13.2^oC$, respectively, in different treatments. The temperature differences in the soil at 15 cm depth beneath the plastic mulch as compared to bare soils ranged from 0 to 9.4^oC for average maximum and 0 to 3.7^oC for average minimum temperatures (Table 1). Higher temperature differences were observed in the soil at 15 cm depth underneath plastic as compared to bare soil, especially in the plots where canola meal cake was applied without mustard (Table 1). The temperatures in the greenhouse for some treatments showed a similar trend as in the field, but were $0\text{-}3^oC$ higher than the field observations for most treatments.

Soil nutrient analysis

The soil pH, organic matter, and nitrate (kg/ha) ranged from 7.6 to 8.0, 1.6 to 1.8, and 99.7 to 817.7, respectively in the field soil. The concentration of nutrients in soil was not significantly different within main plot, sub-plot and sub-sub-plot except for a few treatment combinations. A significantly higher soil pH was observed in the mustard-applied mulch treatment as compared to the control (nothing applied, no mulch), and the rest of the combinations were not significantly different from each other. The concentration of phosphorus (P), potassium (K), calcium(Ca), Magnesium(Mg), manganese (Mn), copper (Cu), boron (Bo), and sulfur (S) by base saturation method in soils were not significantly different among different treatments. However, zinc (Zn), iron (Fe), and magnesium (Mg) were significantly different in some combinations tested by the same base saturation method (Table 2). Non-significant mean data are not presented.

Effect of bio-fumigation on incidence and severity of fungal soil-borne pathogens

In the current studies, Fusarium basal rot caused by *Fusarium oxysporum* f. sp. c*epae* and Pink Root caused by *Phoma terrestris*, were the most commonly observed fungal diseases in the field and in greenhouse bioassay studies. Fusarium basal rot usually occurred on scattered plants or in localized areas throughout the field. No foliar symptom of Fusarium blight, as a unilateral or general wilt and a yellow to tan dieback of leaf tips during mid to late season, was observed in the field. However, rotting of roots and gray to brown discoloration was commonly observed. In addition, storage decay, mostly symptoms as that of Fusarium basal rot infection, were also observed. Other possible causes such as stem and bulb nematodes were not investigated, despite the higher numbers of these nematodes observed in soil samples. Chicken manure did not reduce the Fusarium basal rot incidence (Table 3). However, significantly lower Pink Root severity ratings were observed in onion roots in the plots with chicken manure applications irrespective of mustard application or plastic mulch (Table 3).

Effect of bio-fumigation on nematode community

Fungal feeding nematodes were predominant over bacterial feeding and free living nematodes, irrespective of the treatment applied (data not presented). In Delta County, Colorado, nine plant parasitic nematode (PPN) genera; *Ditylenchus dipsaci* (Onion bulb rot nematode), *Pratylenchus penetrans* (root lesion), *Meloidogyne* (root-knot nematode), *Aphelenchoides*, *Helicotylenchus*, *Hoplolaimus*, *Xiphinema*, *Paratylenchus* and *Trichodorus*, were found associated with onion crops (Table 4). *Pratylenchus* populations were significantly lower in solarized plots in all combinations except in the mustard plus canola treatments. Similarly, *Pratylenchus* populations in plots applied with mustard plus chicken manure were significantly lower as compared to plots without mustard. No *Pratylenchus* populations were observed in the plots treated with mustard, chicken manure and solarization; this was significantly lower than the same treatment combination without solarization. Similarly, no *Meloidogyne* populations were observed in plots with mustard plus chicken manure, irrespective of soil solarization. The nematode populations within chicken manure applied plots were significantly lower in solarized as compared to non-solarized plots (Table 4). Although the data are not presented from greenhouse studies, the same trend followed on treatment effect but the numbers were a bit higher than the field study.

Effect on onion yield

No significant difference among different treatment combinations was observed in the onion bulb yield in medium, jumbo and total yield components per plot (data not shown). Similarly, there was no significant difference in mean bulb size in jumbo and total number of bulbs. However, the onion bulb size in chicken manure applied plots without plastic mulch was significantly larger than the same treatment with mulch (data not shown). The remaining combinations were not significantly different from each other.

Effect on storage quality
The onion bulbs in storage started sprouting in March, and sprouting reduced the onion quality. In this experiment, 61.9 to 74.6% of bulbs did not sprout and were fit for human consumption. There was no effect of treatment on onion sprouting quality in storage. The percentage of rotten bulbs in storage ranged from 8.5 to 18.8% with the lowest percentage of rotten bulbs observed in mustard plus plastic mulch treatment and the highest in chicken manure without plastic mulch (Data not shown).

DISCUSSION

In onion, fungal soil-borne pathogens have been extensively studied in the past but only limited information is available on plant parasitic nematodes (PPNs). However, PPNs may be equally or more important as they reduce the crop yield and predispose plants to infection by other soil-borne pathogens. They do not produce specific aerial symptoms in most cases. Nematode infection remains unnoticed and is often confused with other symptoms caused by nutrient deficiencies or viral infection unless plants are carefully uprooted and observed for root symptoms such as lesions or galls. But not all PPNs produce such diagnostic root symptoms in different crops. Higher *Pratylenchus* and *Meloidogyne* nematode populations were found in Colorado onion fields in addition to association of other nematode genera with a lack of distinct aerial or root symptoms (Pokharel *et al*., 2009). Management options targeted to one pest may affect others as they coexist in soil and may infect single plants together in a field.

Thus, this study was designed to evaluate the efficacy of combined use of bio-fumigation, chicken manure and soil solarization on the control of field populations of major fungal and nematode pathogens prevalent in western Colorado. The primary goal was to evaluate the effect of mulching to reduce the escaping toxic gas released from decomposing mustard green, canola meal cake and chicken manure by the use of plastic mulch where mustard was grown after sweet corn harvest, incorporated at 45 days, followed by soil solarization during 19 September to 31 October, 2008. The secondary goal was to fit these practices on existing growers' crop rotation system. However, soil solarization during this time of year appeared to not be most effective against soil-borne pathogens for western Colorado conditions, as Pokharel and Larsen (2008) found mid-June to late July to be more appropriate for soil solarization.

No effect of soil solarization (mulch) on fungal diseases such as Fusarium basal rot and Pink Root was observed in either the greenhouse or field studies. This result does not agree with Katan *et al*. (1990) who found a significant reduction in Pink Root incidences in onion by soil solarization in Israel. The difference may be due to several factors such as location (temperature, soil type, and crop history), time of experiment, and pathogen populations. We observed only 28.3°C in the present study, whereas Katan *et al*. (1990) observed 59.6^{o}C in their experiment. In the current experiment, the highest temperature observed does not agree with Pokharel and Larsen (2008) who recorded a maximum temperature of 43.4^{o}C for an extended period in solarized soil in western Colorado. The difference may be because this experiment was conducted during September to October and their experiment was conducted during June to July.

In the current studies, a significant reduction in *Pratylenchus* and *Meloidogyne* nematode populations by soil solarization was observed. These results agree with several other studies done in the past. Populations of *Pratylenchus thornei* were greatly suppressed by soil solarization (Greco *et al.*, 1990). Similarly soil solarization for 15–30 days provided satisfactory control of root-knot nematode (Giannakou *et al*., 2007). For effective soil solarization, temperatures inside plastic commonly should exceed 35°C for an extended period of time depending on soil depth and duration. Many soil pests are adequately controlled by 4-8 weeks of soil solarization in the upper 10-30 cm (rooting zone).

The current study showed that the plastic mulch increased soil temperature and that was influenced by soil amendments. In another experiment at WCRC-Orchard Mesa site, mustard plus plastic mulch raised soil temperatures up to 54.0^{o}C as compared to soil solarization alone at 42.4^{o}C (Pokharel, unpublished). The higher temperature obtained inside mulch with soil amendments is believed to produce a higher concentration of volatile compounds which otherwise would have escaped into the environment. The concentrations of many volatile compounds emitted from decomposing organic materials released into the soil atmosphere have been shown to be significantly higher when the amended soil was solarized (Gamliel and Stapleton, 1993). Moreover, combining these materials with solarization can sometimes greatly increase the biocidal activity of the amendments (Stapleton, 2000).

Several species of *Brassica* are known to have antimicrobial volatile compounds such as allyl isothiocyanate in various forms in their leaf extracts (Mayton *et al*., 1996) and also other sulfur-containing compounds (Sang *et al*., 1984). However, such information on the efficacy of bio-fumigants on Fusarium basal rot and or Pink Root is limited. In our current studies (field and greenhouse), bio-fumigation was not effective against Fusarium basal rot or Pink Root. These results do not agree with other findings on several other pathogens. Snapp *et al*. (2004) found that incorporation of oriental mustard (*Brassica juncea* L. variety Pacific Gold) in the spring before planting potatoes suppressed *Rhizoctonia solani* by 73%, and the plastic cover was highly suppressive for fungal activity of *Pythium ultimum*, and *Fusarium solani*. The difference in results could be due to difference in the species of fungi associated, variety of *Brassica* used, season of the experiment, etc. The fungus species involved, the growing season, and incorporation time in the present study were different than Snapp *et al*. (2004) despite the use of *Brassica, B. juncea* L., variety Pacific Gold in both experiments. In the present study, *Fusarium oxysporum* f. sp. c*epae* was the target species whereas Snapp *et al*. (2004) targeted *Fusarium solani;* in addition, mustard was grown and incorporated in the fall in the present study versus Snapp *et al*. (2004) who used a spring planting.

The prevailing temperature during decomposition might have affected the breakdown process by release of volatile gas and its efficacy against fungal pathogens. In addition soil clods formed in the present study due to higher soil moisture by irrigation prior to incorporation of organic matter might have contributed to lack of efficacy. The release of isothiocyanate from decomposing tissue may be slow and/or low during fall, and/or big clods might have reduced gas circulation inside the soils. It might be possible that the species of *Brassica* used was not effective against *Fusarium oxysporum* f. sp. c*epae* pathogen; as the efficacy varies with fungus and crop species, and with the age and type of *Brassica* tissue which might have influenced the type and concentration of isothiocyanates released (Kirkegaard *et al*., 1999). This study also does not agree with Blok *et al*. (2000) who found an effective control of soil-borne pathogens by applying fresh organic amendments followed by mulching.

In the current studies, bio-fumigation by mustard alone was effective in reducing the nematode populations of *Pratylenchus*, *Meloidogyne* and *Helicotylenchus* in both field and greenhouse tests. These results agree with several other studies done in the past on the efficacy of isothiocyanate on different nematode species. Even rotational plantings of rape or mustard in strawberries checked the spread of some nematodes (Winkler and Otto, 1980), particularly *Pratylenchus penetrans*. Similarly, growing and incorporating rapeseed in soil significantly reduced populations of *Meloidogyne chitwoodi* (Mojtahedi *et al*., 1993), *M. incognita* and *M. javanica* (Johnson *et al*., 1992). In the presence of myrosinase, glucosinolates are toxic to *Heterodera schachtii (* (Lazzeri *et al*., 1993). However, the level of efficacy might be different due to types or species of *Brassica* used, species of nematode targeted, and concentration of isothiocyanate released from the mustard where Wu *et a1*. (2009) reported a direct relationship between isothiocyanate concentration and disease control.

Addition of manures to the soil enhanced soil suppressiveness of fungal pathogens such as *Rhizoctonia solani* (Martinson, 1995). However, no such information is available on the effect of chicken manure on Fusarium basal rot and Pink Root in onion. It was expected that the application of chicken manure would increase soil suppressiveness and plant nutrients, especially micronutrients, thereby increasing onion yield. The successful control of root-knot nematodes by amending soil with chicken manure, thought to be based on the release of toxic levels of ammonium, alterations in soil structure, the stimulation of antagonistic organisms, and improved plant tolerance (Lazarovits *et al*., 2001), has been well documented.

In our current studies a significant reduction in *Meloidogyne* populations was observed by chicken manure application. The efficacy of chicken manure on *Meloidogyne* and other pathogens could be increased in western Colorado and under similar conditions by performing this process during June or July when the temperature is higher. Lopez-Perez *et al*. (2005) reported that efficacy of these soil amendments including chicken manure is temperature dependent; chicken manure was more effective in controlling *Meloidogyne incognita* in greenhouse tomato at 30^{o} C than at 20 or 25^{o}C. In addition, manure that is high in nitrogen and soluble (available) carbon has been shown to suppress soil-borne pathogens (Tenuta and Lazarovits, 1999) and improve plant health due to reduction in pathogen populations (Lazarovits, 2001).

Soil amendments such as chicken manure, mustard green, and canola meal cakes also aid plant nutrients in addition to bio-fumigation effect. The increased availability of mineral nutrients following soil solarization include those in the organic fraction, such as NH_4-N, NO_3-N, P, Ca, and Mg, as a result of the death of the micro-biota (Stapleton *et al.,* 1990). In the current study, application of chicken manure alone, and mustard alone increased nitrate N by 300 kg/ha and 118 kg/ha, respectively which were lower than their combination of both at 3767 kg/ha. The liberation of N compounds (vapor and liquid) is a component of the mode of action. Increased concentration of reduced N would then nitrify after termination of soil solarization to provide NO_3 for increased crop growth (Stapleton *et al.,* 1991). But that did not happen in our study possibly due to the difference in amount of these amendments, composition of such materials, and temperatures. In addition, the bulb size and mean plot yield were not significantly different among different treatments. Continued application of such organic matter through plant and animal sources might help build up the organic matter and release micronutrients through several repeated applications, while in our current studies we used such materials only once. The result of this study was not the expected reduction in Pink Root incidence and increased onion yield as in the case of Telon 17 (Schwartz, 1986) who reported a significant reduction in Pink Root infection and increased onion yield by the chemical fumigant.

These organic materials, such as animal manure and crop residues, could be combined with soil solarization to enhance bio-fumigation by increasing soil temperature through decomposition of these materials and also by increasing the heat carrying capacity of the soil (Gamliel and Stapleton, 1993)**.** Organic amendments, particularly plant residues and animal manure, augment the biocidal activity of bio-fumigation through the production of biotoxic volatile compounds emanating from the decomposition of organic materials (Gamliel and Stapleton , 1993)**.** Treatment of soil with organic and inorganic NH_4- based fertilizers and soil solarization were effective against natural populations of *Pythium ultimum* and *Meloidogyne incognita* in the soil. Composted chicken manure alone at 5381 kg/ha significantly reduced *Pythium* sp., and when combined with heat (42°C), the *Pythium* population was eradicated (Stapleton *et al.*, 1991). In the current studies, chicken manure reduced the incidence of Pink Root but not Fusarium basal rot. This result was similar to that of Aryantha *et al*. (2000) who reported that chicken manure amendments, whether composted or not, also reduced populations of *P. cinnamomi* after 12 weeks and increased seedling viability. Application of fresh chicken manure was highly effective in reducing the incidence of potato scab, *Verticillium* wilt and parasitic nematode populations (Conn and Lazarovits, 1999).

Combinations of soil solarization with chicken manure, mustard meal cake and mustard green manure might have greater impact on soil-borne pathogens. Exposure to sub-lethal heating weakens the pathogens leading to reduction in viability and to susceptibility to other biological and physical control mechanisms (Katan and Devay, 1991). The numbers of *P. ultimum* and *S. rolfsii* propagules were reduced by more than 95% when the exposed to volatile compounds from heated cabbage or compost amended soil for 14 days (Gamliel and Stapleton, 1993). Significantly, the highest reduction of *Pratylenchus* populations was observed in mustard, chicken manure and solarization combinations where significantly lower *Pratylenchus* populations were observed in chicken manure and solarization combinations. Blok *et al*. (2000) found an effective control of soil-borne pathogens by fresh organic amendments followed by mulching. In our study, a significant reduction was also observed in *Meloidogyne* in chicken manure with mustard combination. Similarly the nematode populations were reduced by the combined use of chicken manure and solarization. Current results of different combinations being effective to reduce *Pratylenchus* and *Meloidogyne* populations were similar to findings of Benlioglu *et al*. (2005) who reported that the effectiveness of chicken manure on soil-borne problems is increased by combining with soil solarization.

Nematodes can play different roles in the soil: beneficials as nutrient cyclers and soil health indicators, potential pathogens (yield reducer), and as agents predisposing plants to other soil-borne fungal pathogens. The composition of nematode communities (plant-parasitic and free-living) may be used as soil health bioindicators (Neher, 2001). In these experiments, higher percentages of PPN (74 to 91%) were observed as compared to beneficial nematodes indicating a poor soil health condition of this field as the healthy soil should contain less than 50% of PPNs and more than 50% of beneficial nematodes (Neher, 2001). However, the percentages of PPNs decreased in the plots treated with chicken manure and soil solarization, thereby improving the soil health of treated soils.

CONCLUSION

Combined use of mustard, chicken manure and plastic mulch increased the efficacy of treatments even though the seasonal timing of the experiment was too late to raise the soil temperature to a level detrimental to most soil-borne pathogens. Some of the treatment combinations showed an impact on onion soil-borne pathogens, especially Pink Root and nematodes such as *Pratylenchus, Meloidogyne,* and *Helicotylenchus.* Management of these pathogens is important for successful onion production. However, a follow up study on treatment timing when the temperature is high might show bio-fumigation and soil solarization to be effective possible alternatives to manage soil-borne pathogens in onion and many other crops. The results indicated that it may not be possible to have very effective bio-fumigation treatments in grower's fields unless they modify their current crop rotation and farm operation systems. Thus further studies are needed to examine application of different soil amendments combined with soil solarization during summer season to see if their efficacy can be enhanced.

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Table 1. Average of maximum and minimum temperatures (^{o} C) in bio-fumigated plots beneath the plastic mulch and in bare soil at a depth of 15 cm (6”) in Delta, CO during 2009.

Main Plot	Sub-plot	Sub-sub-plot	Averages Minimum	Averages Maximum
Mustard	Canola Meal Cake	No mulch	8.3	19.3
		Mulch	7.6	22.5
	Chicken Manure	No mulch	6.9	27.5
		Mulch	9.5	27.7
	Control	No mulch	9.2	25.9
		Mulch	10.2	29.9
No Mustard	Canola Meal Cake	No mulch	10.0	17.0
		Mulch	9.8	26.4
	Chicken Manure	No mulch	10.5	21.2
		Mulch	9.5	22.4
	Control	No mulch	9.5	24.3
		Mulch	13.2	28.3

Table 2. Effect of different bio-fumigation treatment combinations and of soil solarization treatment on soil pH, some plant nutrients by base saturation method in onion soils from Delta, CO. during 2009.

			Soil pH	Nitrate (Kgs)	Fe ppm	Zn ppm	Na ppm	K ppm*	Mg ppm*
Mustard	Canola Meal Cake	No mulch	8.0ab	226.0b	9.6 a	1.7 ab	137.0 ab	2.34a	15.0 ab
		Mulch	8.0 ab	190.0b	8.1 ab	1.5 b	322.0 a	2.0a	14.7 ab
	Chicken Manure	No mulch	7.9 ab	45.0c	8.4 ab	1.6 ab	105.0 ab	2.7a	16.4 ab
		Mulch	7.9 ab	3721.0a	6.9 b	1.6 ab	171.0 ab	2.0a	17.7 a
	Control	No mulch	8.0 ab	30.0c	8.7 ab	1.5 b	79.0 b	2.3a	13.4 b
		Mulch	8.0 a	148.0b	8.7 ab	1.7 ab	140.0 ab	2.3a	14.7 ab
No Mustard	Canola Meal Cake	No mulch	7.8 ab	320.0b	8.6 ab	1.6 ab	160.0 ab	2.7a	16 ab
		Mulch	7.8 ab	201.0b	9.5 ab	1.6 ab	108.0 ab	2.3a	13 b
	Chicken Manure	No mulch	7.9ab	46.0c	8.9 ab	1.7 ab	117.0 ab	2.7a	17.7 a
		Mulch	7.8ab	346.0b	7.8 ab	1.4 b	147.0 ab	2.3a	17 a
	Control	No mulch	7.6b	162.0b	8.4 ab	1.8 ab	145.0 ab	2.3a	14.7 ab
		Mulch	7.8ab	58.0c	9.1 ab	2.08 a	117.0 ab	2.3a	16.4 ab

Note: means with same letter are not significantly difference with DMRT P= 0.05 level.
*% Base Saturation

Table 3. Efficacy of bio-fumigation on incidence of onion Pink Root and Fusarium basal rot at pre-harvest and harvest in Delta, CO during 2009.

Main Plot	Sub-plot	Sub-sub-plot	Pink Root Mean severity ratings*	Fusarium basal rot infected bulb numbers	
				Two weeks before harvesting	At harvesting
Mustard	Canola Meal Cake	No mulch	2.0 abcde	2.0 ab	7.0 a
		Mulch	2.4 a	4.0 a	9.7 a
	Chicken Manure	No mulch	1.5 f	3.0 ab	9.7 a
		Mulch	1.5 f	2.0 ab	7.7 a
	Control	No mulch	1.7 def	3.0 ab	11.4a
		Mulch	2.3 ab	1.0 b	5.7 a
No Mustard	Canola Meal Cake	No mulch	2.1 abc	1.0 b	13.0a
		Mulch	1.7 ef	2.7 ab	11.7a
	Chicken Manure	No mulch	1.6 f	2.4 ab	11.0a
		Mulch	1.7 def	2.0 ab	12.3a
	Control	No mulch	2.0 abcde	2.0 ab	8.7 a
		Mulch	1.75 cdef	1.4 ab	9.7 a

Note: Means with the same letter in a column are not significantly different to each other by DMRT test at alpha > = 0.05 level. * average of 25 random plant roots and a scale of 0-10.

Table 4. Effect of bio-fumigation and plastic mulch on the incidence of beneficial nematode numbers and plant parasitic nematode genera and their number in Delta, CO during 2009.

Treatments			Numbers		Percent	Nematode numbers			
Main plot	Sub-plot	Sub-sub-plot	PPNs	Bene fitial nema todes	of plant parasitic nematod es	Dity[a]	Praty[b]	Meloi[c]	Heli[d]
Mustard	Canola Meal Cake	No mulch	230	80.0	74.2	146.7a	33.3b	1.0 e	20.0b
		Mulch	460.0	146.7	75.8	360.0ab	33.3b	20.0 c	26.7b
	Chicken Manure	No mulch	576.7	53.3	91.5	440.0 a	20.0d	0.0 e	33.3ab
		Mulch	380.0	86.7	81.4	253.3ab	0.0 e	0.0 e	26.7b
	Control	No mulch	656.7	200.0	76.7	346.7ab	166.7ab	26.7ab	13.3b
		Mulch	286.7	100.0	74.1	213.3ab	6.7d	0.0 e	26.7b
No Mustard	Canola Meal Cake	No mulch	263.3	133.3	66.4	193.3ab	26.7 c	13.3 c	0.0d
		Mulch	273.3	73.3	78.9	126.7b	13.3d	66.7 a	13.3b
	Chicken Manure	No mulch	500.0	86.7	85.2	193.3b	206.7a	6.7 d	20.0b
		Mulch	466.0	160.0	74.4	380.0ab	53.3 b	0.0 e	0.0d
	Control	No mulch	393.3	180.0	68.6	233.3ab	40.0 b	6.7 d	53.3a
		Mulch	226.7	46.7	82.9	126.7b	6.7 d	6.7 d	53.3a

Note: [a] = *Ditylenchus dipsaci*, [b] = *Pratylenchus penetrans* [c]= *Meloidogyne* spp [d] = *Helicotylenchus.*
PPNs= Plant parasitic nematode numbers, BFN= Beneficial nematodes numbers