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Effect of Calcium Cyanamide Treatment Combined with Different Fertilizers on Root-knot Nematode Disease

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Abstract: The cultivation of chili peppers in Zhanjiang City, Guangdong Province, China, has long suffered from root-knot nematode, which has caused significant economic losses. Commonly used chemical nematicides are highly toxic to humans and animals, and they can remain in the soil, damaging soil health and affecting the cultivation of subsequent crops. There is a need to find a green and safe alternative. Therefore, the present field experiment was conducted based on laboratory studies to evaluate the effect of treatment of open field with lime nitrogen followed by different fertilizers on the control of root-knot nematode in chili and its growth. The highest preventive effect against root-knot nematode was found to be 53.37% after applying lime nitrogen treatment in the open field, and the highest preventive effect was 80.42% when combined with fertilizers. Compared with the control, the maximum increase in soil temperature was 8°C, and the pH value of the soil was increased from 5.83 to 7.52, effectively improving soil acidification and structure and increasing soil fertility. The present results show that this technology can meet the expectations when applied on the open floor, but it needs to be combined with other measures. It can be carried out in combination with the summer fallow period.

1. Introduction

Capsicum annuum L. is a plant of the genus Solanaceae. As one of the oldest plants planted in human history, it originated in Mexico and was introduced into China during the Ming Dynasty. As a kind of condiment, *Capsicum annum* L. is deeply loved by the whole country because of its unique flavor[1-3]. China is the world's largest producer and consumer of chili, and the domestic and foreign consumption demand for chili is increasing year by year; the cultivation of chili has become an important economic source for farmers in some areas to get rid of poverty and get rich[4,5]. *Meloidogyne* spp. is a plant-parasitic nematode that can infest a variety of crops, causing crop quality decline, poor root vitality, yield reduction, and other problems, ranging from weak growth and small plant type to whole plant wilt and death[6,7]. In addition, it can be spread along with the transportation of vegetable seedlings, insect-bearing soil, irrigation water sources, and agricultural operations, which has a very adverse impact on the development of the pepper industry[8-10].

DiWang(DW) is a new type of plant-derived organic medicinal fertilizer made from the fermentation of Bacillus amylolytic HN11 by spreading tobacco waste as the substrate, which has a control effect of 90.89% against tomato root nematode and a good control effect on rice, sweet potato, strawberry and other crops' pests and diseases[11,12]. GenZhongKang(GZK) is mainly composed of Bacillus subtilis and Bacillus gelatine. Studies have shown that these two types of microorganisms can enrich the microbial population in soil and have an excellent inhibition effect on root-knot nematodes[13-16]. Calcium Cyanamide, a kind of alkaline nitrogen fertilizer, has been applied in China's agricultural field for more than 100 years[17]. Calcium Cyanamide is mainly used to disinfect greenhouse soil, carried out using solar high-temperature confinement sheds. It can provide nutrients for soil as a base fertilizer, increase soil pH value, improve soil acidification, improve soil structure, and improve soil fertility. It can inhibit the occurrence of root-knot nematodes[18-20]. The temperature in the shed can reach more than 70℃, and the temperature at 15 cm of the ground under the film can still get more than 40℃ [21-23], and it is rarely applied on the open field. Studies have shown that the pesticide action of calcium cyanamide is mainly through its decomposition process to produce cyanamide substances, which can effectively inhibit and kill underground pests such as root-knot nematodes, pathogens, and weeds[24-26]. In this study, the open field was treated with Calcium Cyanamide, covered with black mulch film, and combined with sunlight exposure to explore the effect of Calcium Cyanamide disinfection, chemical fertilizer, and bacterial fertilizer on the control and growth of root-knot nematode of capsicum.

2. Materials and methods

2.1 Overview of the test sit

The experiment site was set up in the pepper planting base of Qianshan Town, Xuwen County, Zhanjiang City, Guangdong Province (20°23 '25.52 "N, 110°27' 52.64" E, altitude 16 m). The local climate is tropical monsoon, with an average high temperature of 33℃ and an average low temperature of 26℃ during the experiment. The tested soil was compact sandy soil. The ground is acidic; pH value is 5.21; Organic matter content was 5.04 g kg^{-1}, available nitrogen 28.67 mg kg^{-1}, available phosphorus 40.26 mg kg^{-1}, available potassium 86.37 mg kg^{-1}. The previous crop was pepper, and root-knot nematodes occurred seriously in the experiment. Multiple community soil samples were measured, with approximately 450~600 insect populations per 100 grams of soil.

2.2 Test materials

Calcium Cyanamide (containing more than 50% Calcium Cyanamide, more than 20% nitrogen (N) element content, more than 38% calcium (Ca) element content, pH 12.4, black-gray granules) (provided by Ningxia Darong Industrial Group Co., LTD.), DW (tobacco residue organic fertilizer, organic matter content ≥45%, South China Agricultural University, Provided by Guangzhou Green Wind Biotechnology Co., LTD.), GZK (Bacillus subtilis · Bacillus gelatinae, adequate viable bacteria number ≥10.0 billion /ml, Handan Jianhua Plant Pesticide Factory), the test pepper variety is spicy crinkle 111_{F1} (Shandong Shouguang Xinxinran Horticulture Co., LTD.)

2.3 Test treatment

Different contents of Calcium Cyanamide were scattered on the surface, and rotary tillage was carried out with a rotary tiller; after mixing, a drip irrigation belt was laid, the film was covered, and water was poured through the drip irrigation system under the film (the amount of Calcium Cyanamide was divided into 600, 900 and 1200 kg ha^{-1}), 25 days after the film was covered, the air was ventilated for three days, and then DW and GZK were applied, and pepper seedlings were transplanted. A total of 16 treatments were set up in this experiment, and each treatment was randomized to a block design. Each treatment was repeated three times. The plot area was 10 m^2, the row spacing of pepper plants was 30 cm * 40 cm, and the row spacing between the plots was set up. The experiment involves 16 treatments, each with three replicates. As shown in Table 1. The dosage of DW was 4500 kg ha^{-1}, and GZK was 75 L ha^{-1}.

Table 1: Calcium Cyanamide mixed with different fertilizers for treatment.

Group	Handle	Dosage
A1	$Ca(CN)_2$-L	600 kg ha^{-1} $Ca(CN)_2$
A2	$Ca(CN)_2$-M	900 kg ha^{-1} $Ca(CN)_2$
A3	$Ca(CN)_2$-H	1200 kg ha^{-1} $Ca(CN)_2$
ck	ck	ck
B1	$Ca(CN)_2$-L+DW	600 kg ha^{-1} $Ca(CN)_2$+4500 kg ha^{-1} DW
B2	$Ca(CN)_2$-M+DW	900 kg ha^{-1} $Ca(CN)_2$+4500 kg ha^{-1} DW
B3	$Ca(CN)_2$-H+DW	1200 kg ha^{-1} $Ca(CN)_2$+4500 kg ha^{-1} DW
ck1	ck+DW	ck+4500 kg ha^{-1} DW
C1	$Ca(CN)_2$-L+GZK	600 kg ha^{-1} $Ca(CN)_2$+ 75 L ha^{-1} GZK
C2	$Ca(CN)_2$-M+GZK	900 kg ha^{-1} $Ca(CN)_2$+ 75 L ha^{-1} GZK
C3	$Ca(CN)_2$-H+GZK	1200 kg ha^{-1} $Ca(CN)_2$+ 75 L ha^{-1} GZK
ck2	ck+GZK	cK+75 L ha^{-1} GZK
D1	$Ca(CN)_2$-L+DW+GZK	600 kg ha^{-1} $Ca(CN)_2$+4500 kg ha^{-1}DW+75 L ha^{-1} GZK
D2	$Ca(CN)_2$-M+DW+GZK	900 kg ha^{-1} $Ca(CN)_2$+4500 kg ha^{-1}DW+75 L ha^{-1} GZK
D3	$Ca(CN)_2$-H+DW+GZK	1200 kg ha^{-1} $Ca(CN)_2$+4500 kg ha^{-1}DW+75 L ha^{-1} GZK
ck3	ck+DW+GZK	ck+4500 kg ha^{-1} DW+75 L ha^{-1} GZK

Note: The experiment involves 16 treatments, each with three replicates. L: low, M: Medium, H: High.

2.4 Investigation contents and methods

2.4.1 Changes in soil pH value and soil temperature

Soil pH value was extracted by deionized water with a water-to-soil ratio of 5:1 and measured by pH meter.

The 5, 10, and 20 cm soil temperature at soil depth was recorded at 8:00, 14:00, and 19:00 during the film coating treatment and measured by a fixed-length metal soil thermometer.

2.4.2 Incidence of root-knot nematode

Six pepper plants were randomly investigated at 70 days in each treatment plot according to the five-point sampling method. The plants were uprooted, the soil on the roots was shaken off, washed with running water, and excess water was sucked up with paper towels. The disease index and relative prevention effect were calculated.

Disease index (%) =∑ (number of each disease grade × number of corresponding grades)/(total number of investigated plants × highest investigated disease grade index) *100

Relative prevention effect (%) = ((control disease index) - (treatment disease index))/control disease index *100

Disease classification was conducted according to GB/ T19780.38-2000 "Guidelines for Pesticide Field Efficacy Trials (I) Nematocides for Control of root nematode disease."

Grade 0: root no gall;Grade 1: There are a few small galls in the root system;Grade 3: 2/3 root system covered with small gall;Grade 5: Roots covered with small galls and secondary galls;Grade 7: The roots form fibrous roots.

2.5 Test data processing

Statistical data analysis was performed using Excel 2019 and SPSS 24.0 software, and Duncan's new complex range (DMRT) method was used for the statistical analysis of experimental data. When $p < 0.05$, the difference could be regarded as significant.

3. Result

3.1 Influence of Calcium Cyanamide Treatment on Soil pH Value

As shown in Figure 1, the pH value of soil treated with different contents of Calcium Cyanamide increased the most from day 3 to day 6. Compared with the control group, the pH value of soil treated with Calcium Cyanamide increased significantly, and $Ca(CN)_2$-L rose from 5.58 to 6.30. $Ca(CN)_2$-M increased from 5.57 to 6.46; $Ca(CN)_2$-H rose from 5.83 to 6.54. The pH value of the control group fluctuated within the range of 5~6 during the whole treatment period. Compared with the control group, the pH value of the three concentration treatments in each group tended to rise slowly from the 9th day, with the highest pH value reaching 7.08, 7.31, and 7.52, respectively, showing an apparent increasing trend.

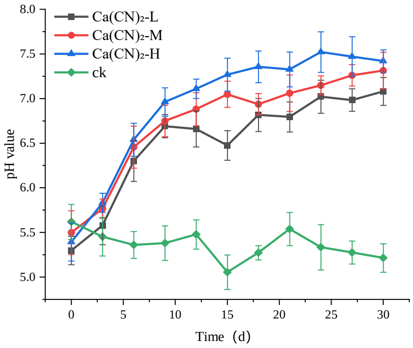

Figure 1: Effect of different concentrations of Calcium Cyanamide treatment on soil pH value.

3.2 Influence of Calcium Cyanamide treatment on soil temperature

It can be seen from Table 2 that the change of soil temperature under different Calcium Cyanamide coating treatments will increase with the increase of application amount. Compared with ck (override) and ck (exposing), the highest temperature of low, medium, and three concentrations at the depth of 5 cm in the soil can reach 42.8℃, 43.0℃ and 46.9℃ respectively, which is 1.6, 1.8 and 5.7℃ higher than ck (override) and 3.9, 4.1 and 8℃ higher than ck (exposing), respectively. ck (override) is 2.3℃ higher than ck (telling). When the soil depth is 10 cm, it is 0.6, 0.7, and 2.2℃ higher than ck (override), 3.6, 3.7, and 5.2℃ higher than ck (exposing), and 3℃ higher than ck (override). When the soil depth is 20 cm, it is 0.8, 1.9, and 3.1℃ higher than ck (override), 2.9, 4, and 5℃ higher than ck (exposing), and 2.1℃ higher than ck (override). The average and maximum temperature of the soil treated with Calcium Cyanamide were higher than that of the untreated group, and the temperature would increase with the increase in the amount of Calcium Cyanamide.

Table 2: Effect of Different Concentrations of Lime Nitrogen Treatment on Soil Temperature.

Soil depth	Deal with	Average temperature for each period (℃)			average annual temperature(℃)	highest temperature(℃)
		8:00	14:00	19:00		
5 cm	$Ca(CN)_2$-L	36.27±0.63c	41.21±1.28c	37.55±1.09c	38.34±1.00ab	42.8
	$Ca(CN)_2$-M	37.44±0.86b	42.45±1.18b	38.83±0.64b	39.57±0.89ab	43.9
	$Ca(CN)_2$-H	38.31±0.66a	45.27±1.91a	40.16±0.67a	41.25±1.08a	46.9
	ck(override)	35.76±0.73c	40.57±0.44c	36.33±1.00d	37.55±0.72ab	41.2
	ck(exposing)	34.03±0.41d	38.16±0.55d	35.67±0.37d	35.95±0.44b	38.9
10 cm	$Ca(CN)_2$-L	34.96±0.58b	40.28±0.69b	38.88±0.71b	38.04±0.66ab	41.6
	$Ca(CN)_2$-M	36.72±0.46a	40.73±0.70b	38.75±0.53b	38.73±0.56ab	41.7
	$Ca(CN)_2$-H	37.09±1.09a	42.11±0.81a	39.81±0.49a	39.67±0.80ab	43.2
	ck(override)	33.86±0.71c	39.58±0.93c	37.49±0.93c	36.98±0.86ab	41
	ck(exposing)	33.45±0.36c	37.27±0.68d	36.75±0.91d	35.82±0.65b	38
20 cm	$Ca(CN)_2$-L	34.78±0.61b	40.32±0.32b	39.06±0.79b	38.04±0.57ab	40.6
	$Ca(CN)_2$-M	35.25±0.56b	41.14±0.45a	39.19±0.47b	38.53±0.49ab	41.7
	$Ca(CN)_2$-H	36.99±0.68a	41.20±0.98a	40.43±0.64a	39.54±0.77ab	42.7
	ck(override)	33.17±1.02c	38.63±0.53c	37.22±0.97c	36.33±0.97ab	39.8
	ck(exposing)	32.51±0.32d	36.94±0.50d	36.27±0.78d	35.24±0.53b	37.7

Note: The data in the table are Mean ± SE, n=3, and analyzed using DMRT (P0.05). The same letter after the data column indicates no significant difference between the groups.

3.3 Control the effect of Calcium Cyanamide combined with different fertilizers on root-knot nematodes

The specific results are shown in Table 3. The increase in Calcium Cyanamide dosage will enhance the overall control effect. There was no significant difference in the control effect of A1, A2, and A3 on root-junction nematodes by a single application of Calcium Cyanamide, which was 47.83%, 50%, and 54.35%, respectively. There was no significant difference between B1, B2, and B3 treated with Calcium Cyanamide combined with DW and single treatment at low concentrations, but there was a significant difference at high concentrations. There was no significant difference between B1, B2 and B2, B3, and the control effect was 58.7%, 60.87% and 65.22%, respectively. Calcium Cyanamide with GZK treatments C1, C2, and C3 did not differ significantly from lime nitrogen medium and high concentration treatments at medium and low concentrations. The differences were significant in the case of high concentration, and the differences were not significant between C1, C2 and, C2, C3 when comparing the groups 61.69%, 63.04%, and 67.39%, respectively. The effects of Calcium Cyanamide combined with DW + GZK treatment were 67.39%, 72.83%, and 80.43%, respectively. Overall, the low-concentration treatment had no significant difference compared with B3 and C3, but the high-concentration treatment had a significant difference compared with other treatments. In summary, with the increase in Calcium Cyanamide dosage, the disease index will show a downward trend, and the control effect will be improved. Calcium Cyanamide soil disinfection treatment combined with DW + GZK had the best effect, and the control effect of each treatment was enhanced with the increase of the amount of Calcium Cyanamide so that the pepper root-knot nematode disease could be suppressed to a certain extent.

Table 3: Effect of different concentrations of lime combined with different fertilizers on root-knot nematode control

Number	Sickness inde(%)	Relative efficacy(%)	Sickness inde(%)	Relative efficacy(%)
A1	39.65±2.75f	45.53±2.32f	C1	30.16±5.50cdef
A2	36.51±2.75fg	49.45±8.34ef	C2	25.40±2.75bcde
A3	33.33±5.50defg	53.37±15.03cdef	C3	23.81±8.25bc
ck	73.02±7.27h	/	ck2	28.57±0.00bcdef
B1	34.92±2.75defg	52.09±1.82def	D1	25.40±2.75bcde
B2	28.57±4.76bcdef	60.88±5.08bcde	D2	19.84±3.64ab
B3	25.40±7.27bcd	65.48±7.33bcd	D3	14.29±2.38a
ck1	34.92±5.50efg	52.25±5.27def	ck3	26.98±2.75bcdef

Note: The data in the table are Mean ± SE, n=6, and analyzed using DMRT (P0.05). The same letter after the data column indicates no significant difference between the groups.

4. Discussion

Calcium Cyanamide soil disinfection, as an efficient and environmentally friendly agricultural technology, has been widely used at home and abroad in recent years. It obtained the official pesticide registration certificate in 2011 and became an advanced and applicable technology promoted by the Ministry of Agriculture in 2014[27]. This technology is mainly used for soil disinfection treatment in indoor greenhouses, and the working conditions require airtight, high temperature, and water, which can effectively reduce the occurrence of soil diseases and pests [23,28]. A large number of studies have shown that Calcium Cyanamide can neutralize acidic substances in soil, and with the increase in the amount of Calcium Cyanamide, the pH value increases more significantly[29,30]. It is especially suitable for application in acidic soil in southern China, which can achieve soil disinfection and improve the ground. However, the warming effect of Calcium Cyanamide mulching on the open field is not as good as that in the confined space of a greenhouse. The maximum temperature detected in the test at 5 cm near the ground reaches 46.9℃, and the lethal temperature of nematodes reaches 42℃[8]. Studies have shown that Wenchang, Hainan, can make the temperature at 5 cm of the soil reach 73.0℃ by using Calcium Cyanamide mulching on the open field [22]. There is a big gap in this study. This also shows that the effect of Calcium Cyanamide application in the open field is highly affected by climate, and it should be combined with other measures to raise soil temperature further so that Calcium Cyanamide can play a role in the open field. The results showed that there was still room for improvement in the control effect of Calcium Cyanamide granules on root-knot nematode disease. The control effect of Calcium Cyanamide granules on root-knot nematode disease was up to 54.35% without any treatment, 65.22% with DW treatment, and 67.39% with GZK treatment, and the control effect of Calcium Cyanamide granules on root-knot nematode disease was up to 80.43% when both were added. Therefore, when the soil is relatively poor or acidified, and the occurrence of root-knot nematodes is rather severe, it is recommended to use Calcium Cyanamide for soil fumigation treatment and combine other measures according to the characteristics of the soil in different places to assist the efficacy of Calcium Cyanamide, to achieve the purpose required for local control.

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