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RESEARCH ARTICLE

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A bio-product as alternative to methyl bromide for replant disease control on strawberry

Abstract Pre-plant soil fumigation with methyl bromide (MB) is a standard practice for controlling soil-borne diseases especially for strawberry diseases. However, the application of MB will be forbidden in China in the year 2015. For this reason, a bio-product named Kangdi 3 was tested as an alternative to MB in strawberry greenhouses in Mancheng (Hebei Province) and Donggang (Liaoning province), China in 2005 and 2006. Methyl bromide at a normal dosage of 500 kg/hm^2 and Kangdi 3 at three dosages of 750, 1500 and 2250 kg/hm^2 were tested. Plots without any treatment were used as the control. During the growing stage, assessments were made on fungal communities in rhizosphere, growth status of strawberry, the disease levels on roots as well as the yields. Results showed that Kangdi 3 significantly reduced the quantity of fungi and the disease index, while enhancing strawberry growth and the yields compared with the untreated control. Therefore, Kangdi 3 is a great potential substitute for methyl bromide to control replant diseases in strawberry.

Keywords soil-borne diseases, strawberry, biological control

1 Introduction

China's strawberry cultivation area has reached 77300 hm^2 and the output has surpassed 1.69 million tons, which is the highest in the world (Zhang et al., 2005). Replant diseases caused by pathogens, such as *Verticillium* spp., *Rhizoctonia* spp. and *Fusarium* spp., are the major obstacles in strawberry production (Zhen et al., 2003; 2005). Application of methyl bromide (MB) to agricultural soils before planting has been the basic method to control nematodes, soil-borne pathogens and weed for many years in strawberry nurseries (Julien and Manker, 2005). The chemistry and air pollution potential of this fumigant as well as its ability to destroy stratospheric ozone have been extensively documented. The use of MB in China will be banned in 2015 according to the Montreal Protocol. However, the current annual consumption of methyl bromide as soil fumigant to control replant diseases in strawberry has surpassed 500 tons, which accounts for about a quarter of the total usage in the area of agriculture and most of the consumption is in Hebei and Liaoning Provinces, China (Tan et al., 2003).

At present, some chemicals such as chloropicrin, metham sodium and dozomet have been reported as alternatives to methyl bromide for controlling soil-borne diseases. One major concern is that these products, while they can be used to substitute for methyl bromide, could cause a new risk to the environment. Therefore, lots of people are looking for bio-products for controlling soil-borne diseases. In this experiment Kangdi 3, a bio-product produced by Qinhuangdao Leading Science and Technology Development Company, China, was tested in greenhouses as a potential substitute for methyl bromide to control strawberry replant diseases.

2 Materials and methods

2.1 Materials

Ninety-eight percent methyl bromide was manufactured by a Sino-Israeli Joint Venture, Lianyungang Dead Sea Bromine Compounds Co., Ltd. Kangdi 3 was produced by Qinhuangdao Leading Science and Technology Development Co., Ltd. Polyethylene (PE) plastic film was produced by Hebei Baoshuo Co., Ltd. Strawberry varieties were Darselect, a late-maturity variety, planted in greenhouses in Mancheng, Hebei Province and Zoji, an early-maturity variety, planted in greenhouses in Donggang, Liaoning Province, China.

2.2 Methods

2.2.1 Treatments and plot design

Two greenhouses located in Mancheng (Hebei, China) and another two in Donggang (Liaoning, China) were used for

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the experiments. Strawberries were continuously cultivated in the fields for at least six years prior to the experiment. A randomized complete block design with four replications was made in each location. The size of each plot was 25 m^2 (7.5 m × 3.3 m). The distance between rows was 0.8 m. The separation distance between plots was 0.4 m. Table 1 showed the names and dosages of the seven treatments in the trials. K1, K2 and K3 represented the three different dosages of Kangdi 3 product.

Table 1 Experimental treatments in Mancheng and Donggang

treatment	product	dosage /kg · hm^{-2}
CK		
MB	Methyl bromide	500
K1	Kangdi 3	750
K2	Kangdi 3	1500
K3	Kangdi 3	2250
1/2MB	Methyl bromide	250
1/2MB + K3	Methyl bromide, Kangdi 3	250 + 2250

2.2.2 Field management

In Mancheng, methyl bromide treatments were made on July 22, 2005. One week after fumigation, the plastic film was removed for evaporation of the remaining MB from the soil. All the other treatments were carried out on August 19. Kangdi 3 was put on the surface of soil followed by ploughing of soil and covering the soil surface with plastic film. On August 22, strawberry seedlings were transplanted into each plot. The greenhouse was covered by plastic film on December 2. During the whole winter, the temperature inside the greenhouse was maintained by covering mats over the greenhouse from 4 p.m. to 8 a.m. the next day.

In Donggang, methyl bromide treatment was carried out on August 21, 2005. The method was the same as in Mancheng. After one week of fumigation, the plastic film was removed on August 29. The other treatments were carried out on September 8. Transplanting was done on September 10. The greenhouses were covered with polyethylene film on October 18.

The other field management such as spraying fertilizers on leaf surface, fumigation of sulfur to prevent powdery mildew and putting a box of bees for flower fertilizing were no different with the local regular management.

2.2.3 Quantitative assessment on soil fungal population

At the beginning of November 2005, populations of soil fungi were estimated in each plot as colony forming units (CFU) in per gram of dry soil. Five samples were randomly selected around the strawberry plants from 10 to 20 cm depth in each plot and mixed to make a composite sample (Fang et al., 1998). All soil samples were air dried at room temperature in open plastic bags. Ten-gram soils were suspended in 90 mL of sterile distilled water in 200 mL flasks and shaken for 30 min at 150 r/min; 100 to 10000-fold dilutions were made by adding distilled water to the suspension above, and 25 mL aliquots from each diluted suspensions were spread onto six potato-dextrose agar (PDA) medium amended with 0.5 g/L streptomycin sulphate (PDAs). Three replicates were performed for each treatment. Plates were incubated at 25°C and after three to five days, colonies were counted (Lacey et al., 1980).

2.2.4 Investigation on the growth, root diseases and yields of strawberry

At the end of November, the height, width and fresh weight of canopy as well as the number, the length and the fresh weight of roots were measured. For these data, 50 plants were taken randomly from each plot. The occurrence of root diseases in Mancheng and Donggang were investigated on March 15 and 30, respectively, in 2006. For each plot, ten plants were dug out from five sites. The roots were cleaned by water first and then the disease severities were investigated based on the following standards (Zhen et al., 2005).

Grade 0: no disease;

Grade 1: diseased area was less than 1/4 of the total roots;

Grade 2: diseased area was between 1/4 to 1/2 of the total roots;

Grade 3: diseased area was between 1/2 to 3/4 of the total roots;

Grade 4: diseased area was over 3/4 of the total roots.

Disease index

$$= \frac{\sum \text{grade level} \times \text{the number of plants on this level}}{\text{the maximum level} \times \text{total number of plants}} \times 100.$$

At each time of harvest, the yield of each plot was measured and accumulated until the last harvest.

2.2.5 Data analyses

The analysis of all the data was performed by using the DPS (data processing system) software version 7.0 for Windows. Differences among treatments were identified with Duncan's multiple-comparison test.

3 Results

3.1 Fungal population in different treatments

Table 2 showed that a significant reduction in soil fungal populations was observed in all treatments, compared with the untreated check (CK). Colony number of each treatment was significantly less than that of each control. However, K3 treatment had the best control effect on the soil fungi with only 1.44×10^5 CFU/g and 1.22×10^5 CFU/g colony number

Table 2 Soil fungal population of different treatments

treatment	number of colony /CFU · g^{-1} × 10^{5}	
	Mancheng	Donggang
CK	3.46 a	3.11 a
K1	2.48 b	2.21 b
K2	2.45 b	1.91 bc
1/2MB	2.07 b	1.72 c
K3	1.44 c	1.22 d
MB	1.40 c	1.19 d
1/2MB + K3	1.03 c	0.96 d

Note: Data are the mean of three replicates measured at × 10^{-4} dilution. The same letter in each column shows there is no significant difference according to Duncan's test at $P = 0.05$.

tested from the treated plot of Mancheng and Donggang, respectively. The colony number of K3 treatment was at the same level as that of MB and 1/2MB + K3.

3.2 Investigation on the growth of strawberry

The results presented in Tables 3 and 4 indicate that there were significant differences among treatments. Both in Mancheng and Donggang, the six items including plant height, plant width, root number, root length, root weight, and canopy weight showed the same trend. After being treated by MB and Kangdi 3, strawberry grew significantly better than the untreated check. In general, the treatments of 1/2MB + K3, MB and K3 showed the best performance for all the items. Except the weight of canopy, treatment of MB and K3 had no significant differences in the other five items. The other three treatments K1, K2 and 1/2 MB had not much difference compared with the untreated check.

3.3 The influence of different treatments on root disease

Figures 1 and 2 show the disease index on roots of strawberry in Mancheng and Donggang, respectively. Although the disease levels were quite different between Mancheng and Donggang, it followed the same trend for all the treatments. From the two figures, it is easy to see that CK treatments had the highest disease index in Mancheng (85.5) and Donggang (28.8), followed by K1, K2 and 1/2MB. The other three treatments K3, MB and 1/2MB + K3 had the same disease level, which was significantly less than that of the above four treatments. Both trials in two locations showed that the Kangdi 3 at the dosage of 2250 kg/hm^2 could inhibit the occurrence of the root diseases with the similar effect as methyl bromide.

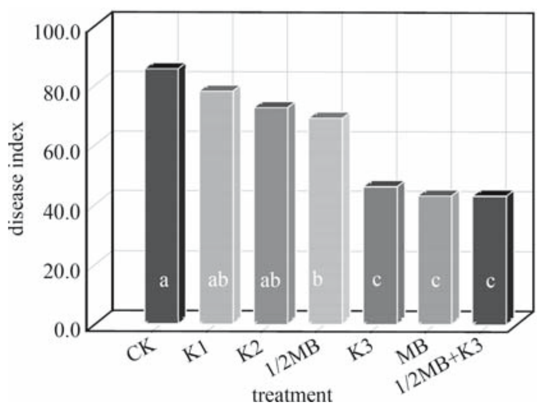

Fig. 1 Root disease index of strawberry under different treatments in Mancheng

Table 3 Physiological status of strawberry under different treatments in greenhouses in Mancheng

treatment	plant height/cm	plant width/cm	number of roots	length of roots /cm	weight of roots /g	weight of canopy /g
CK	5.9 c	16.5 d	12.2 c	13.1 b	3.7 e	15.5 d
K1	7.1 bc	18.9 c	13.1 bc	13.4 b	5.2 de	21.6 cd
K2	7.1 bc	17.1 d	13.8 abc	13.4 b	5.7 bcd	20.5 cd
1/2MB	6.8 c	17.0 d	13.6 abc	14.6 ab	5.6 cd	22.6 c
K3	8.8 a	20.8 b	18.1 a	17.6 a	7.3 bc	45.8 b
MB	8.1 ab	20.5 bc	17.4 ab	16.2 ab	7.6 ab	59.2 a
1/2MB + K3	9.0 a	22.9 a	16.2 abc	16.0 ab	9.3 a	52.4 ab

Table 4 Physiological status of strawberry under different treatments in greenhouses in Donggang

treatment	plant height/cm	plant width/cm	number of roots	length of roots /cm	weight of roots /g	weight of canopy /g
CK	16.4 d	18.3 d	12.2 c	13.1 b	3.7 e	15.5 d
K1	16.8 cd	18.6 cd	13.1 bc	13.4 b	5.2 de	21.6 cd
K2	17.8 bcd	20.2 bcd	13.8 abc	13.4 b	5.7 bcd	20.5 cd
1/2MB	17.9 bcd	20.3 bcd	13.6 abc	14.6 ab	5.6 cd	22.6 c
K3	18.5 b	20.7 b	18.1 a	17.6 a	7.3 bc	45.8 b
MB	18.4 bc	20.4 bc	17.4 ab	16.2 ab	7.6 ab	59.2 a
1/2MB + K3	20.5 a	23.1 a	16.2 abc	16.0 ab	9.3 a	52.4 ab

Note: Each value in Tables 3 and 4 is the average of four replicates, and each replicate represents the average value of 50 samples. The same letter in each column shows there is no significant difference according to Duncan's test at $P = 0.05$.

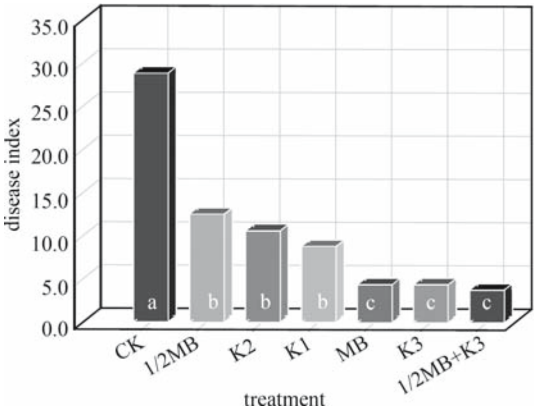

Fig. 2 Root disease index of strawberry under different treatments in Donggang

3.4 Yields of strawberry

After maturing, the strawberry fruits in two locations were harvested from time to time. The yield in each plot was recorded each time. Table 5 shows the accumulative yields of each treatment in Mancheng and Donggang. In general, the variation of yields between treatments in Donggang was not as much as that in Mancheng. In Donggang only the yields in K3 and 1/2MB + K3 treatments were significantly higher than the untreated control. The low variation in treatments could be explained by the low diseased level in the trial field. The yields in Mancheng could be divided into three groups. 1/2MB + K3, MB and K3 were at the first level followed by 1/2MB, K2 and K1. All the treatments were significantly better than the untreated control.

Table 5 Strawberry yields in Mancheng and Donggang under different treatments

Mancheng		Donggang	
treatment	average yield / $kg \cdot hm^{-2}$	treatment	average yield / $kg \cdot hm^{-2}$
1/2MB + K3	31840 a	K3	28000 a
MB	30840 a	1/2MB + K3	26880 a
K3	29680 a	K2	25040 ab
1/2MB	17560 b	K1	23760 ab
K2	16360 b	1/2MB	22080 ab
K1	15080 b	MB	21440 ab
CK	8800 c	CK	18720 b

Note: Each value is the average of four plots of the same treatment. The same letter in each column means there is no significant difference according to Duncan's test at $P = 0.05$.

3 Discussion

Potential causes of the replant disease in strawberry include nematodes, fungi, bacteria, and plant nutrition. It is likely that different problems or combinations of problems are involved in the different strawberry planting areas, and they all do great harm to China's production of strawberry. The replant disease has been a serious problem for the sustainable production of strawberry under continuous cropping.

Soil fumigation before planting is often recommended to control replant disease, although it has never been known exactly what in the soil causes the problem (Liu et al., 1999). Because traditional chemical methods do great harm to the environment, as a pollution-free and sustainable developmental method, biological control is preferred by more and more people. Many studies used biological methods to control the replant diseases by using antagonistic microorganism, mycorrhizal fungi and suppressive soil but with less success in practice (Handelsman and Stabb, 1996). Because lots of factors limit the proliferation of antagonistic microbes in the soil, it is difficult for antagonistic microbes to play their role in most situations. Kangdi 3 is composed of not only a strong antagonistic bacterium but also three kinds of natural medicinal plant materials. These materials, on one hand, had a strong inhibitive effect against the growth of the three pathogenic fungi. On the other hand, they served as the growth media for the antagonistic bacteria (Song et al., 2005). The experiments in the two locations elucidated that when Kangdi 3 was used at a dosage of 2250 kg/hm^2, it had a strong inhibitive effect against the population of fungi and disease level on roots. At the same time, it enhanced the strawberry growth and the yields. Kangdi 3 exhibited a great potential as alternative to methyl bromide for replant disease control in strawberry production. Currently, the limitation for extending the use of Kangdi 3 in large areas lies on its high cost. This problem could be solved with the further improvement of processing procedure and artificial cultivation of medicinal plant materials. Although it will take some time for the successful substitution of methyl bromide in real practice, an attractive prospect has been found.

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