Influence of pH, Sodium and Potassium Salts on Viability of Resting Spores of *Plasmodiophora brassicae*

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ABSTRACT

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The viability of resting spores of *Plasmodiophora brassicae* was detected by using the method of fluorescence microscope and staining with vital dyes of acridine orange and calcofluor white M2R. Observations on the effect of temperature on the viability of resting spores made by Takahashi & Yamaguchi are confirmed. After 48 hr in 0.01 M concentrations of Na_2CO_3, K_2CO_3, NaOH and KOH at pH higher than 10, the viability of the resting spores decreased from 70% to 23, 25, 5 and 25%, respectively. Sodium and potassium carbonate solutions were found to decrease viability of resting spores at lower pH level than sodium or potassium hydroxide solutions. When concentrations of Na_2CO_3 concentration were increased to 0.1 M, viability of resting spores decreased significantly, but at the same higher level of concentration, $CaCO_3$ had no effect. Na_2CO_3, K_2CO_3 and pH levels higher than 10 seem to be the major factors causing the decrease in viability of resting spores.

Key words: *Plasmodiophora brassicae*, resting spore, viability, pH, Na_2CO_3, K_2CO_3.

INTRODUCTION

Suppressive soils of clubroot of crucifers (*Plasmodiophora brassicae* Woronin) are restricted to certain areas in Taiwan. Hsieh & Wang (5) showed that these soils had a higher pH value (> 7.4) and a higher calcium (1210 ppm) content. Wang & Hsieh (12) found that addition of 0.1% Na_2CO_3 to conducive soil to increase soil pH from 5.8 to 7.6 decreased the clubroot disease index considerably from 73 to 0. This significant reduction is due to either soil pH (2,9,12,13) or ions of Ca^{+2} (2,3,5,6,9,12,13), Na^+ or CO_3^{-2} or both having an effect on the resting spores in the soil. The purpose of this investigation is therefore to verify the effect of soil pH and ions of Ca^{+2}, Na^+, CO_3^{-2} on the viability of the resting spores of *P. brassicae* by the fluorescence microscope technique developed by Takahashi & Yamaguchi (10-11).

MATERIALS AND METHODS

Preparation of resting spore suspension of *P. brassicae*

Clubbed roots of cabbage were collected from the fields at Nantou Hsien and the roots with resting spores of *P. brassicae* were blended with a suitable volume of distilled water in a juicer and filtered through cheese cloth (5,6,12). Resting spore suspension was diluted to 1×10^7 spores/ml distilled water.

Staining and examining the viability of resting spores

Spore suspensions were heated in a water bath at 100 C for 1 hr, stained by vital dyes (4): 0.005% acridine orange (4,14), 0.01% calcofluor white M2R and 0.1% evans blue, and examined under fluorescence microscope (Nikon Labophot, mercury lamp, HBO-100w/2) with B and V excitation filters (main wavelengths wer 480 and 405 nm, respectively). Dead spores are stained and the viable spores are not stained by this method. Cytoplasm of heat-killed resting spores will stain orange-red by acridine orange, and blue by calcofluor white M2R (Fig. 1). Evans blue (4) can also stain cytoplasm of heat-killed resting spores. A clear distinction between cytoplasm and cell wall can be made by using two separate stains: acridine orange was used to stain cytoplasm of damaged and killed resting spores and calcofluor white M2R to stain cell wall.

The experiments were conducted at room temperature. Four replications in each treatment and 200-300 resting spores in each replication were stained and examined.

Effect of temperature on resting spores of *P. brassicae*

Spore suspensions were heated between 30-100 C in a

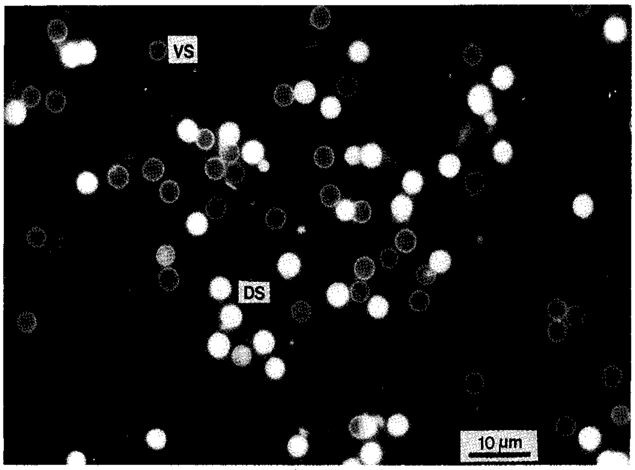

Fig. 1. Cytoplasm of damaged or dead resting spores (DS) of *Plasmodiophora brassicae* stained by calcofluor white M2R under fluorescence microscope. Viable spores (VS) did not stain.

water bath at 10 C intervals for 10 min, stained and examined to determine their viability by the method of Takahashi & Yamaguchi (10,11).

Effect of carbonates on viability of resting spores

Spore suspensions were added in 0.01 M amounts of Na_2CO_3, K_2CO_3, $CaCO_3$ or $NaHCO_3$ solutions, stained and examined for viability at 12 hr intervals, respectively.

Effect of sodium and potassium salts on viability of resting spores

In addition to Na_2CO_3 and K_2CO_3, other sodium and potassium salts were also used to ascertain the influence of sodium and potassium ions on the viability of the resting spores. Other salts used include Na_2CO_3, $NaHCO_3$, Na_2SO_4, $NaNO_3$, Na_2HPO_4, NaH_2PO_4, CH_3COONa, NaOH, NaCl, K_2CO_3, $KHCO_3$, K_2SO_4, KNO_3, K_2HPO_4, KH_2PO_4, CH_3COOK, KOH, KCl. Spore suspensions were added in 0.01 M amounts without adjusting the pH, and examined for viability after 48 hr. Control was treated with distilled water. In order to determinate the effect of pH on viability, spore suspensions were added to Na_2CO_3 and K_2CO_3 solutions with pH adjusted from 8.5 to 11 at 0.5 pH intervals by 0.1 N NaOH or KOH and 0.1 N HCl, and examined after 48 hr. Control was treated with 0.01 M NaOH and KOH at the same pH level.

Effect of concentrations of Na_2CO_3 and $CaCO_3$ on viability of resting spores

In preliminary test, treatment of 0.01 M of Na_2CO_3 decreased the viability of the resting spores (Fig. 3), while the same amount of $CaCO_3$ treatment did not have any effect on the viability. The concentrations of Na_2CO_3 and $CaCO_3$ were therefore increased to 0.1 M in 0.01 M amounts to determine any further effect on the resting spores, and the resting spores examined after 48 hr after treatment in each of these concentrations.

RESULTS

Effect of temperature on viability of resting spores

Viability of resting spores decreased from 87% at 30 C to 5% at 70 C, and resting spores were killed at 80 C

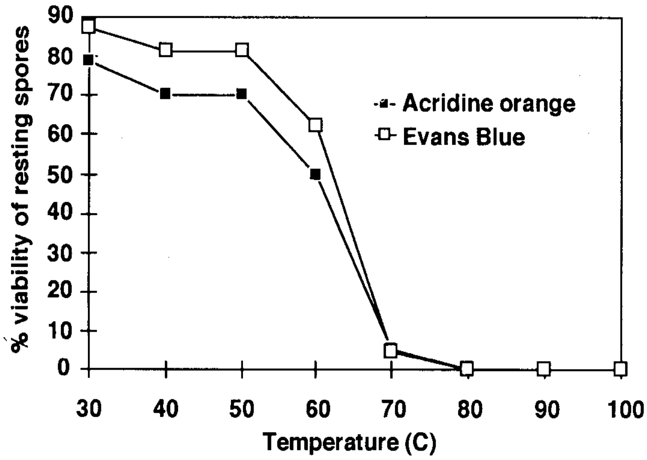

Fig. 2. Effect of temperature on viability of resting spores of *Plasmodiophora brassicae.* Spore suspensions were heated in a water bath from 30 to 100 C for 10 min, respectively, then stained by 0.005% acridine orange and 0.1% evans blue. Dead or damaged spores were stained orange-red by acridine orange and blue by evans blue, viable spores were unstained.

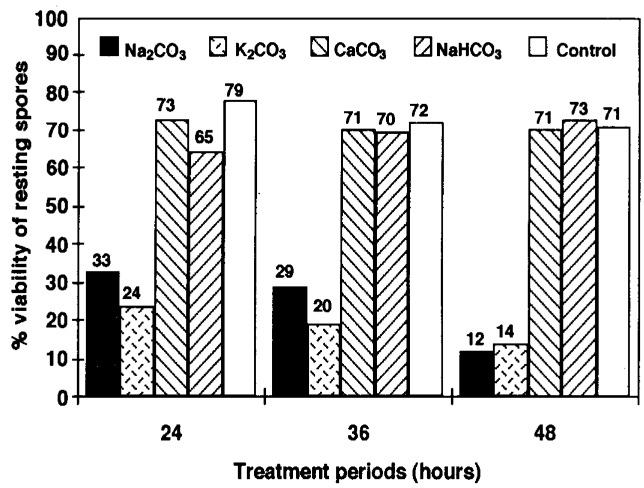

Fig. 3. Effect of 0.01 M carbonates on viability of resting spores of *Plasmodiophora brassicae* at room temperture for 24, 36, 48 hr, respectively. Numbers above bars show the percentage of viability of resting spores in each treatment.

when exposed for 10 min (Fig. 2). A similar observation on the effect of temperature on viability of *P. brassicae* resting spores was made by Takahashi & Yamaguchi (10,11).

Effect of carbonates on viability of resting spores

Na_2CO_3 and K_2CO_3 treatments decreased viability of resting spores from 71% to 12 and 14%, respectively after 48 hr. On the other hand, $CaCO_3$, $NaHCO_3$ and distilled water treatments had no effect on the viability of resting spores (Fig. 3).

Effect of sodium and potassium salts on viability of resting spores

Na_2CO_3, NaOH, K_2CO_3 and KOH solutions with pH higher than 10 decreased viability of resting spores from 70% to 23, 5, 25 and 25%, respectively (Fig. 4). At pH 10, Na_2CO_3 and K_2CO_3 decreased viability of resting spores from 71% to 7 and 12%, respectively, but at the same pH level, NaOH and KOH had no effect. At pH 11, NaOH and KOH decreased spore viability from 71% to 16 and 15%, respectively (Fig. 5).

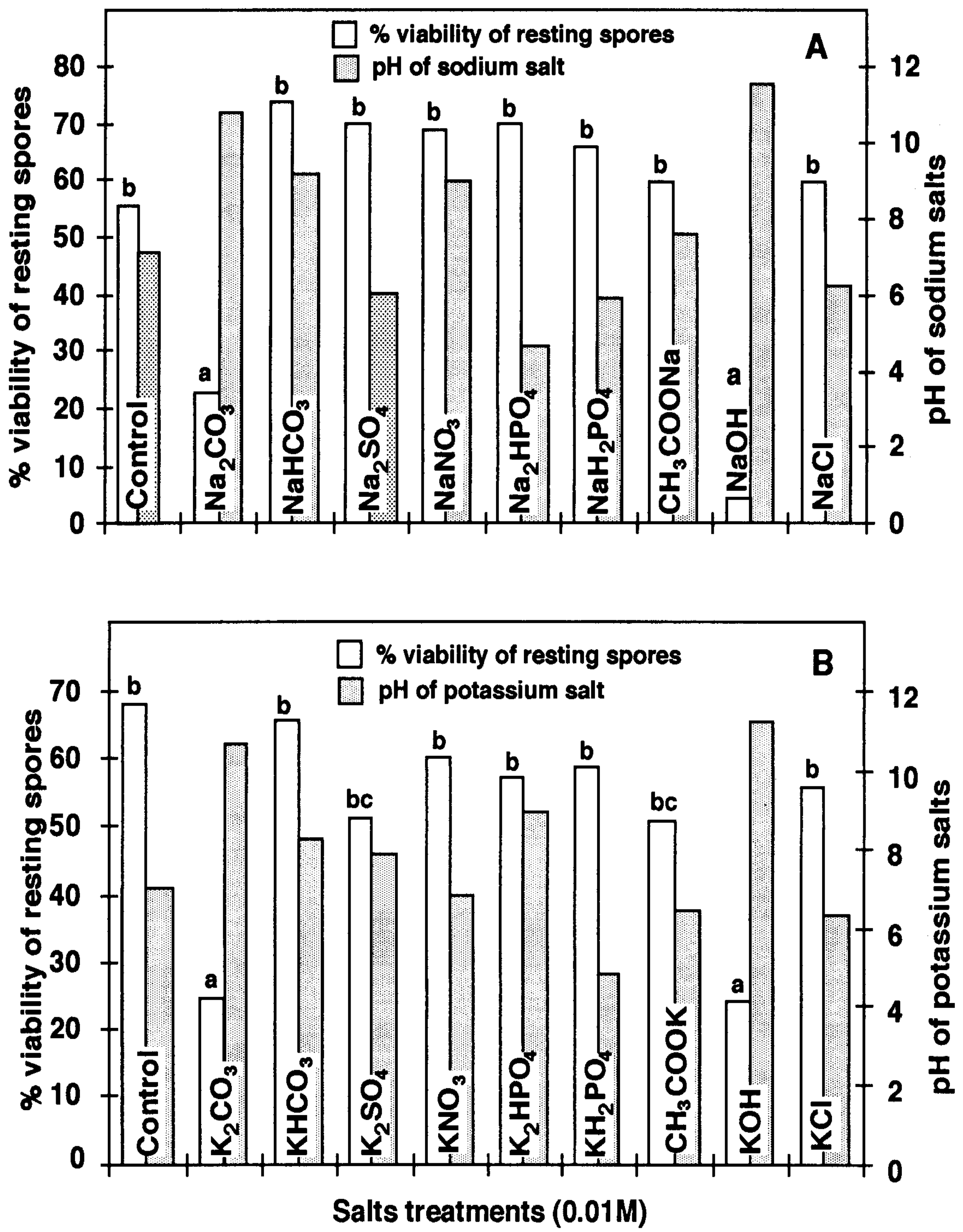

Fig. 4. Effect of sodium salts (A) and potassium salts (B) on viability of resting spores of *Plasmodiophora brassicae* at room temperature after 48 hr. Same letter above bars indicates means were not different according to Scheffle F-test (P = 0.05). Each bar represents the mean of four replications.

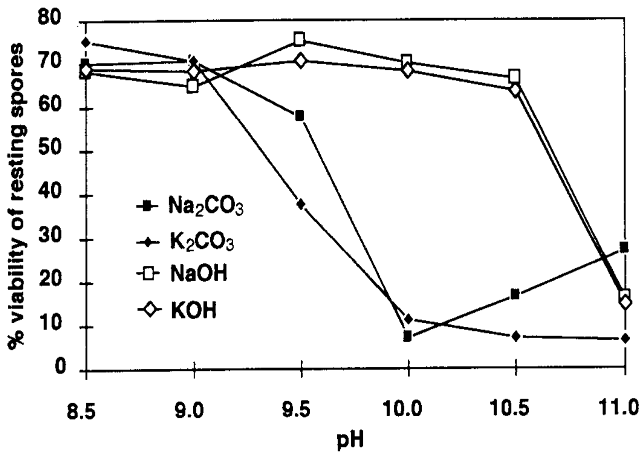

Fig. 5. Effect of Na_2CO_3, K_2CO_3, NaOH and KOH at 0.01 M on viability of resting spores of *Plasmodiophora brassicae* at different pH levels at room temperature after 48 hr. pH of Na_2CO_3 and K_2CO_3 increased by 0.1 N NaOH and KOH, respectively, and decreased by 0.1 N HCl.

Effect of concentrations of Na_2CO_3 and $CaCO_3$ on viability of resting spores

Na_2CO_3 at a concentration of 0.09 M decreased the viability of resting spores from 61% to 0, while the same concentration of $CaCO_3$ had no effect on the viability of resting spores (Fig. 6).

DISCUSSION

Of the carbonates used in our trials, Na_2CO_3 and K_2CO_3 decreased viability of resting spores from 71% to 12 and 14%, respectively. The viability was not affected by $CaCO_3$, $NaHCO_3$ or distilled water (Fig. 3). Cations like Na^+, K^+ and Ca^{+2} form NaOH, KOH and $Ca(OH)_2$ in water and increase pH to a higher level. At pH 10, Na_2CO_3 and K_2CO_3 decreased spore viability from 71% to 7 and 12%, respectively (Fig. 3, 4). NaOH and KOH with pH 11 also decreased spores viability from 71% to 16 and 15%, respectively (Fig. 4, 5). Increasing concentration of $CaCO_3$ to 0.1 M at a pH level of 9.1 had no effect on spore viability, however, increasing the concentration of Na_2CO_3 to 0.1 M at a higher pH level of 11.2 decreased viability from 61% to 0 (Fig. 6). In these trials, pH level higher than 10 and cations seem to be the two most important factors affecting viability of resting spores. The effect of these cations on viability of resting spores has yet to be investigated.

Buczacki & Moxham (1,7,8) report that five layers of resting spore wall with charge and salt linkages are composed of alkaline-soluble proteins, chitins and lipids. We believe that when resting spores are soaked in alkaline solutions, linkages of resting spore wall are broken down gradually, allowing exchange of H^+, Na^+, K^+, CO_3^{-2} ions between cell membrane of the resting spore, thereby affecting the pH level. Cytological study is required to confirm this hypothesis. Suppressive soils have an avarage pH level of 7.4 in Taiwan (5,12) and conducive soils have a lower pH. When alkaline compounds like

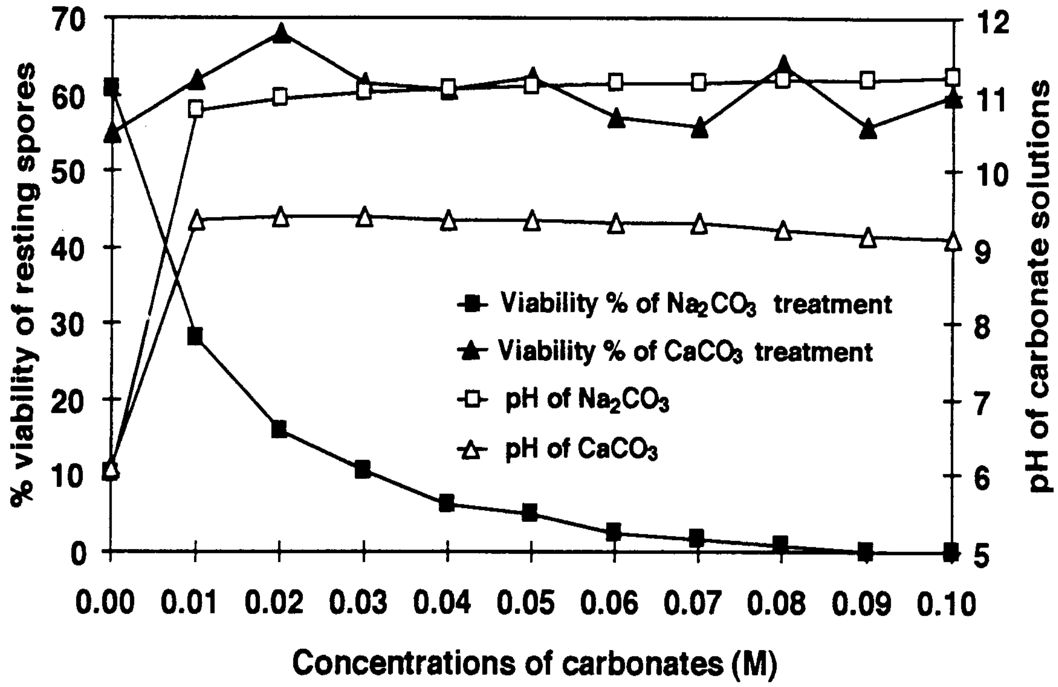

Fig. 6. Effect of molar concentrations of Na_2CO_3 and $CaCO_3$ on viability of *Plasmodiophora brassicae* at room temperature after 48 hr.

Na_2CO_3, K_2CO_3 and $CaCO_3$ are added to conducive soils, they adhere to soil particles containing the resting spores. The capillaries or micropores among soil particles became filled with water when irrigated and the alkaline compounds are dissolved. The irrigation probably increases the pH of the solution in capillaries to a higher enough level sufficient to decrease the viability of resting spores in soil. Previous preliminary trials on the effect of pH on soils (5,6,12) indicate that increase in soil pH from 5.8 to 7.6 do not damage cabbage roots and other trials elsewhere (2,3,8,13) on the effect of pH on soil also confirm our observations.

Although application of NaOH and KOH increased the pH level in soil and decreased viability of resting spores, their use is not recommended because of their corrosive property which may cause structural damage to the soil particles. When applying alkaline compounds like Na_2CO_3, K_2CO_3, $CaCO_3$ etc., factors like the method of application, types of lime used, lime particle size, distribution in soil and soil water contents must be carefully considered (2,3,9,12,13). Effects of these alkaline amendments on soil structure are not fully understood and had to be thoroughly investigated before this method can be safely used to decrease clubroot disease. Although alkaline compounds reduced clubroot disease, they have not been tested in experiments with other soil types.

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摘　　要

李敏郎、謝文瑞. 1992. **酸鹼值、鈉鹽及鉀鹽對十字花科蔬菜根瘤病菌 (*Plasmodiophora brassicae*) 休眠孢子活性之影響**. 植病會刊 1:31-36. (台中市　國立中興大學植物病理學系)

本試驗利用 0.005% acridine orange 及 0.01% calcofluor white M2R 螢光染劑，探討碳酸鹽類、鈉鹽及鉀鹽對十字花科蔬菜根瘤病菌 (*Plasmodiophora brassicae*) 休眠孢子

活性的影響。在30 C時，休眠孢子活性爲87%，若以70 C處理10分鐘，則活性降至5%，而致死溫度爲80 C。休眠孢子以0.01 M碳酸鹽類、鈉及鉀鹽處理48小時後，只有碳酸鈉、碳酸鉀、氫氧化鈉及氫氧化鉀具有降低休眠孢子活性的顯著效果，其活性自對照組的70%分別降至23、25、5及25%；在較低酸鹼值時，碳酸鈉及碳酸鉀比氫氧化鈉及氫氧化鉀較具降低休眠孢子活性的效果。休眠孢子以Na_2CO_3及$CaCO_3$處理48小時後，其活性隨碳酸鈉濃度增加(0.01 M–0.1 M)，而自61%降至0，而以碳酸鈣處理者並無影響。由本試驗得知高酸鹼值(pH ＞ 10)、碳酸鈉及碳酸鉀爲降低休眠孢子活性的主要因子。

關鍵字：十字花科蔬菜根瘤病菌、休眠孢子、活性、酸鹼值、碳酸鈉、碳酸鉀。