Short Research Article

Control Efficacy of Jingdusha Against *Corynesporacassiicola* on Cucumber

ABSTRACT

Aims: The aim of the paper was to clarify the effect of cucumber target leaf spot (TLS) under the Jingdusha (JDS) treatment.

Study design: We applied the method of artificial inoculation in the pot, and analyzed the changes in growth indexes and physiological characteristics.

Place and Duration of Study: In 2018, these experiments were conducted in College of Bioscience and Biotechnology of Shenyang Agricultural University (Lab 240).

Methodology: The seedlings in two-leaf period were induced by the best application scheme of JDS, then inoculated *Corynesporacassiicola*for 24 h. Cucumber seedlings of each treatment group were randomly selected for photographing and growth index determination after inoculation for 5 d. The leaves of cucumber seedlings in each treatment group were randomly collected at 1 d, 3 d, 5 d, 7 d and 9 d after inoculation for the determination physiological and biochemical indicators.

Results: When *C. cassiicola*infects cucumber, JDS can effectively improve the growth and photosynthetic pigment content of cucumber, reduce the degradation of chlorophyll (Chl) under the stress of *C. cassiicola*, strengthen the variety of metabolic responses in the plant, repair the enzyme protection system of cucumber leaves, reduce the accumulation of reactive oxygen species, shorten the process of membrane lipid peroxidation in blades.

Conclusion: Taken together, these results suggest that JDS can improve the resistance of cucumber seedlings to*C. cassiicola* by regulating growth indexes and physiological characteristics. This work will provide a theoretical basis for further elucidating molecular mechanism of JDS in cucumber defense against *C. cassiicola*.

Keywords: Jingdusha; Cucumber; Induced resistance; Corynesporacassiicola; Physiological and biochemical parameters

1. INTRODUCTION

Target leaf spot (TLS) was also called *Rhizoctonia solani*and Cercospora leaf spot, which is caused by *C. cassiicola* [1,2]. TLS has a wide host range and mainly affects cucumbers and tomatoes, which makes the production of cucumber decrease by 20%~70% [3]. In recent years, TLS has become increasingly harmful to cucumber production. We mainly use chemical pesticides to resist TLS in field production. However, the continuous use of pesticides not only makes pathogens resistant but ~~pollute~~pollutes the environment [4-6].

A resistance inducer is a specific microorganism or plant molecule, which can induce plants themselves to stimulate immune systems, with high efficiency, security and sustainability and broad-spectrum [7,8]. Currently, using a resistance inducer to make plants have the ability to resist diseases on their own to defend against some bad conditions has gotten satisfying results [9], but lack of the research on the resistance of cucumber as a host. JDS is a kind of plant immune resistance inducer whose main components are chitosan, bio-iodine, and natural amino acids. Among them, chitosan can effectively improve the disease prevention effect of fruits and vegetables, such as tomato against botrytis cinerea, cucumber against pythium, pumpkin against powdery mildew, potato against*Clavibacermichiganensis*subsp. *sepedonicus* and cotton against *Colletotrichum gossypii*Soutllw. and so on [10,11].

In our previous study, applying JDS as a resistance inducer on the cucumber seedlings can improve the ability to resist TLS. In particular, three times inductions at 7 d intervals for 1 d with 2.0 $mL{\cdot}L^{-1}$ of JDS treatment provided the best protection against the TLS [12]. Nevertheless, the resistance mechanism of JDS on TLS is still not clear. This experiment will adopt the above scheme to spray treatment on tested cucumbers, and then inoculate the pathogen TLS, we tested and analyzed the changes of various growth indexes and physiological characteristics, preliminary clarify the resistance mechanism of TLS under the JDS treatment. This paper discussed the impossible function of TLS treated with a kind of plant resistance inducer JDS, ~~so as to~~to provide an essential basis for better application of resistance inducers in the production of cucumber in the northeast solar greenhouse.

2. MATERIAL AND METHODS

2.1 Materials

The cucumber tested in this study was JinKe 1, which is provided by Tianjin Kexing Vegetable Research Institute. *C. cassiicola* was purchased from the Bacterium Conservation Center of the Chinese Academy of Agricultural Sciences. JDS was purchased from Qingdao Seawin Biotech Group Co., Ltd.

2.2 Experiments Design and Treatment

This experiment began from September to December 2018 in a plant culture room, which is located in College of Bioscience and Biotechnology of Shenyang Agricultural University. After accelerated generation, cucumber seeds were cultivated on pots with 2/3 of turf soil and 1/3 of vermiculite in a light incubation room. Cucumber seedlings of uniform growth were selected when the plants had two leaves and one heart, and were induced every 7 d with 2.0 $mL{\cdot}L^{-1}$ of JDS for a total of three inductions, then inoculated *C. cassiicola* on each treated leaves after the last induction 24 h. The specific method of induction and inoculation followed as described by Meng [12]. There are four treatments in this experiment, (1) cucumber seedlings only treated with spray water as CK group (2) cucumber seedlings induced by JDS as JDS group (3) cucumber seedlings affected with *C. cassiicola*as *C. cassiicola*group (4) cucumber seedlings treated with JDS and *C. cassiicola*as JDS+*C. cassiicola* group. Each group had twenty seedlings and repeated three times.

2.3 Measurement of Growth Index

We chose six plants treated with the method mentioned before to take photos, and another nine plants to measure the growth index after inoculation for 5 days. The height and main root length (mm) of cucumber seedlings were determined by a meter ruler, and the stem thickness was determined by a vernier caliper, the leaf area of the third euphylla of seedlings

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was determined by the square grid method [13], and the fresh and dry weights of the whole seedlings (0.01 g) were determined by an electronic scale, and calculate moisture content and seedling strength index (strength index = (stem thickness/plant height) × whole plant dry weight).

2.4 Measurement of Physiological and Biochemical Index

The third euphylla was harvested at five time points (1, 3, 5, 7, and 9 days post-inoculation [dpi]) to take measures of the physiological and biochemical index, each treatment was replicated three times, and the materials from each treatment were collected in triplicate.

The contents of Chl were determined by Acetone ethanol colorimetric method [14]. The contents of soluble sugar and protein were measured by the anthrone colorimetric method and Coomassie brilliant blue G250. The activity of POD was determined by guaiacol method, SOD activity was determined by the nitroblue tetrazolium method, the activity of CAT and PAL was determined by methods refer to Ulgen [15]. The contents of MDA ~~was~~were determined by the thiobarbituric acid method [16].

2.5 Statistical Analysis

Data was conducted by Microsoft Excel 2010. The ANOVA method of SPSS 17.0 software was applied for the significance of differences. Pictures were created by using Origin 8.5.

3. RESULTS AND DISCUSSION

3.1 Effects of JDS Treatment on Seedlings Growth in Cucumber under *C. cassiicola*Stresses

By comparing with the CK group, JDS treatment promoted the increase in leaf area of cucumber seedlings, had little effect on plant height and stem thickness, and inhibited root length growth (Fig. 1 and Table. 1). Otherwise, the root length and leaf area of cucumber that in *C. cassiicola*group were inhibited remarkably than CK, representatively reduced by 44.0% and 10.70%. Furthermore, JDS+*C. cassiicola*treatment could reduce the inhibition of root length and leaf area of cucumber seedlings infected by *C. cassiicola*, in this condition, the leaf area of cucumber seedlings increased by 9.48% than CK group.

As shown in Table 2, the fresh weight and moisture content of cucumber seedlings treated with JDS respectively increased by 69.67% and 2.77% compared with CK group. It shows that JDS treatment can efficiently improve the moisture content of cucumber seedlings. Compared with CK group, the dry weight and strength index of cucumber seedlings in the *C. cassiicola*group were significantly reduced by 16.22% and 22.67%, while in JDS+*C. cassiicola* group, those two factors increased by 21.62% and 10.67% respectively. It can indicate that the accumulation of biomass in cucumber seedlings under the *C. cassiicola* stress was inhibited and the strength index was also decreased, however, the JDS group got the contrary result.

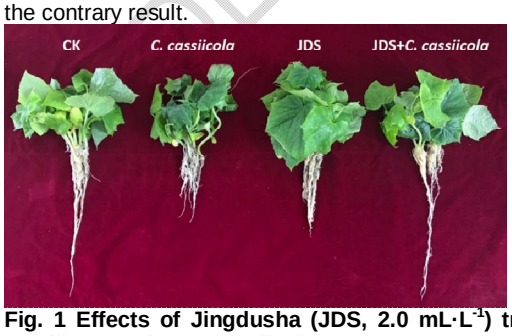

Fig. 1 Effects of Jingdusha (JDS, 2.0 mL·L^{-1}) treatment on the growth of cucumber seedlings under *C. cassiicola* stresses

Table 1 Effects of JDS treatment on the morphological index of cucumber seedlings under *C. cassiicola* stresses

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Treatment	Plant height/cm	Root length/cm	Stem thickness/cm	Leaf area/cm^2
CK	8.25±0.76^{a}	24.50±1.50^{a}	1.65±0.27^{a}	48.21±0.83^{c}
JDS	8.48±1.06^{a}	17.47±0.47^{b}	1.65±0.35^{a}	62.74±0.53^{a}
C. cassiicola	8.01±0.96^{a}	13.50±0.87^{c}	1.52±0.29^{a}	43.05±1.77^{d}
JDS+*C. cassiicola*	8.09±1.29^{a}	23.50±1.41^{a}	1.49±0.29^{a}	52.78±1.11^{b}

^{a,b}*The different letters indicate significant differences according to LSD and Duncan test ($P \leq 0.05$)*

Table 2 Effects of JDS treatment on the biomass and strength index of cucumber seedlings under *C. cassiicola* stresses

Treatment	Fresh weight/g	Dry weight/g	Moisture content/%	Strength index
CK	5.21±0.89^{b}	0.37±0.01^{b}	92.78±1.06^{bc}	0.075±0.004^{a}
JDS	8.84±0.75^{a}	0.41±0.01^{a,b}	95.35±0.28^{a}	0.080±0.003^{a}
C. cassiicola	4.98±0.87^{b}	0.31±0.01^{c}	93.67±0.92^{b}	0.058±0.003^{b}
JDS+*C. cassiicola*	5.52±0.36^{b}	0.45±0.05^{a}	91.87±0.38^{c}	0.083±0.011^{a}

3.2 Effects of JDS Treatment on the Contents of Photosynthetic Pigments in Cucumber Leaves under *C. cassiicola* Stresses

Fig. 2 shows that the content of Chl a, Chl b and carotenoid in the cucumber seedlings are higher than CK group during the entire sampling period, indicating that JDS treatment significantly increased the content of the photosynthetic pigment in cucumber seedlings.

The content of Chl a, Chl b and carotenoid in the cucumber seedlings of the *C. cassiicola*group are consistently lower than the CK group. The content of the photosynthetic pigment in cucumber seedlings of JDS+*C. cassiicola*group is lower than the JDS group, the photosynthetic pigment content in both *C. cassiicola* and JDS+*C. cassiicola* group tended to increase and then decrease with increasing stress time, and both reached a maximum at 3 d after inoculation. The content of Chl a, Chl b and carotenoid in the cucumber seedlings of JDS+*C. cassiicola*group is higher than the *C. cassiicola*group on 5, 7 and 9 days after inoculation. It can be seen that *C. cassiicola*reduces the content of the photosynthetic pigment in cucumber blades. But spraying JDS can increase the photosynthetic pigment to a certain extent, which was conductive to plant adaptation to the stress environment.

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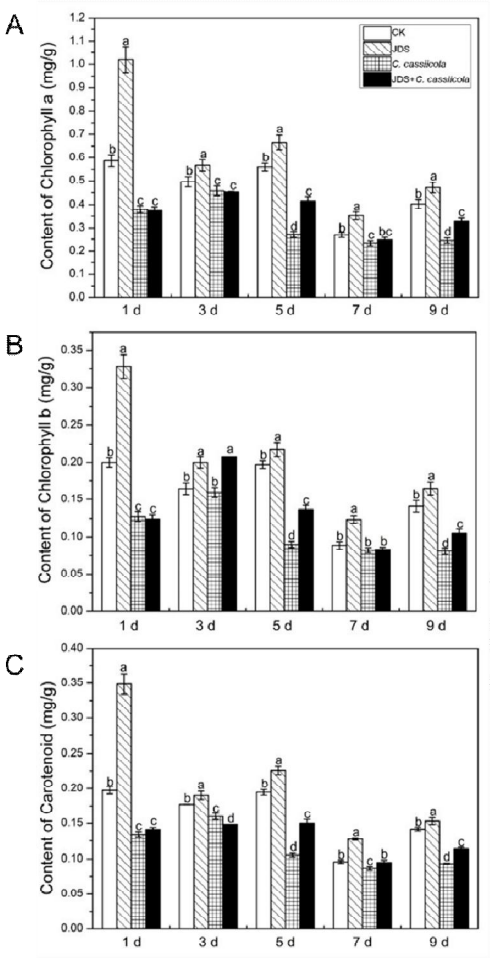

Fig. 2 Effects of JDS treatment on the contents of photosynthetic pigments in cucumber under *C. cassiicola* stresses

3.3 Effects of JDS Treatment on the Contents of Soluble Sugar and Protein in Cucumber Leaves under *C. cassiicola* Stresses

During the whole sampling period, the content of soluble sugar in cucumber seedlings of the JDS group is higher than the CK group, which can represent that JDS can significantly induce the increase of soluble sugar content in plants. The content of soluble sugar in cucumber seedlings of JDS+*C. cassiicola* group was significantly higher than that of *C. cassiicola* at 1 d after inoculation, not significantly different from that of *C. cassiicola* at 3 d, 5 d, and 7 d, and lower than that of *C. cassiicola* at 9 d (Fig. 3A). The content of soluble sugar in cucumber seedlings of JDS+*C. cassiicola* group was significantly higher than that of *C. cassiicola* at 1 d, 7 d and 9 d after inoculation and there is no distinction to the *C. cassiicola*group after 3 d and 5 d inoculation (Fig. 3B).

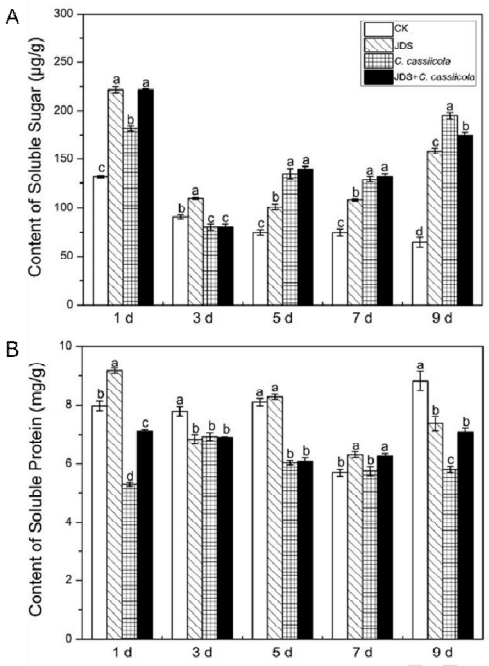

Fig. 3 Effects of JDS treatment on the contents of soluble sugar and protein in cucumber under *C. cassiicola* stresses

3.4 Effects of JDS treatment on the activity of the defense-related enzyme in cucumber leaves under *C. cassiicola* stresses

As shown in Fig. 4A, there are two maximums of the POD activity at 5 d and 9 d in the CK group, but these two are lower than that treated with JDS. The POD activity of JDS treatment showed an increasing trend and reached a peak at 3 d, which was 3.10 times higher than that of CK, indicating that JDS treatment markedly induced an increase in the POD activity of the plants. After *C. cassiicola*affecting 9 d, the statistic of JDS+*C. cassiicola*group is higher than both the JDS group and *C. cassiicola*group.

As shown in Fig. 4B, the activity of SOD has an increasing tendency and that of the JDS group is always higher than the CK group, but the most significant difference appeared at 5 d. In JDS+*C. cassiicola*group, the top level of SOD activity is 7 d after inoculation, but in the *C. cassiicola*group, the maximum is 9 d. Concluding by analyzing these statistics, JDS and *C. cassiicola*can induce the activity of SOD in cucumber seedlings to get to the top level rapidly.

As shown in Fig. 4C, the activity of SOD in the CK group shows a trend from decline to rise, in the JDS group has a rising tendency, both the two groups reach the peak at 9 d, JDS group is 17.22 U g^{-1} min^{-1} higher than CK. In JDS+*C. cassiicola*group, a period of 1 to 7 d is obviously higher than the JDS group and *C. cassiicola* group, the biggest difference is 9 d after inoculation.

As shown in Fig. 4D, when the activity of PAL in cucumber seedlings treated with JDS is significantly higher than the CK group from 5 d, the maximum is 51.22 U g^{-1} min^{-1} at 9 d, which is the 1.35 times than the CK group. In JDS+*C. cassiicola*group the top level of PAL activity is at 1 d after inoculation, but the result in the *C. cassiicola*group is 5 d after

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inoculation, representing that JDS can effectively induce the improvement of PAL activity in cucumber seedlings.

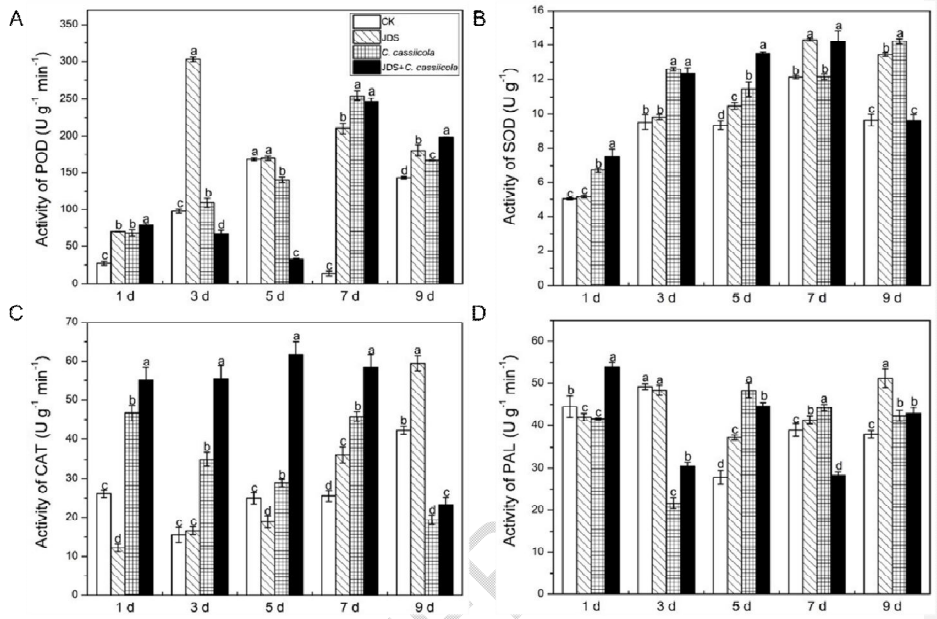

Fig. 4 Effects of JDS treatment on the activity of the defense-related enzyme in cucumber under *C. cassiicola*stresses

3.5 Effects of JDS Treatment on the Contents of MDA in Cucumber Leaves under *C. cassiicola*Stresses

As shown in Fig. 5, the content of MDA in cucumber seedlings treated with JDS is significantly lower than in the CK group, and it keeps a low level throughout the whole sampling period. In the *C. cassiicola*group, the highest level of MDA is the day after inoculation, in JDS+*C. cassiicola*group is 9 d, meanwhile, the content is lower than the *C. cassiicola*group at 3 d and 5 d. These suggested that being treated with JDS can inhibit the process of membrane lipid peroxidation in blades.

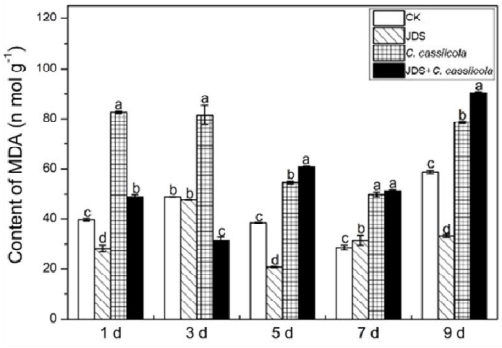

Fig. 5 Effects of JDS treatment on the contents of MDA in cucumber under *C. cassiicola* stresses

4. CONCLUSION

Cucumber growth and development are severely affected by TLS, and it also obviously reduces root length, leaf area and dry weight. Leaf area reduction may be caused by leaf senescence or oxidative damage, and the reduction of root length and dry weight may be caused by the production of virulent pathogenic factors by *C. cassiicola* on photosynthetic organs or plant structures. However, the use of biological resistance inducer solves the inhibition of *C. cassiicola*stresses to cucumber seedlings and increases leaf area, fresh weight and moisture content strikingly, which is advantage for seedlings' development. The strength index is a target for judging plants' growth, the higher the value, the better the plant is growing. When the cucumber seedlings are treated with JDS, the strength index does not differ a lot, but it can relieve the effect of *C. cassiicola* on cucumber seedlings' strength index. Chl is an indispensable role in plant photosynthesis, and it can also affect the usage of plants to light energy, which is a representation of the ability of plant photosynthesis [17]. Under *C. cassiicola* stresses, the content of the photosynthetic pigment in cucumber leaves, but after spraying JDS can relieve the inhibition of *C.cassiicola*to the photosynthetic pigment in cucumber leaves and increase the content of Chl in leaves, which is beneficial to adapting threatening environment.

The production of induced resistance in plants is usually achieved through enzyme-catalyzed regulation, POD, SOD, ~~CAT~~CAT, and PAL are important enzymes in plant resisting reactions, which have become essential factors in judging plant resistance in the process of host-pathogenic bacteria reaction [18]. POD is widely found in plants and is closely related to respiration, photosynthesis and the oxidation of growth hormones [19]. SOD is an important antioxidant enzyme in living organisms with special physiological activity and is the primary substance for scavenging radicals in living. The level of SOD in an organism is a visible indicator of aging and death [20]. CAT is an enzyme cleaner, which can divide H_2O_2 into O^{2-} and H_2O and clean it in plants to protect cells from the damage of H_2O_2 [21]. PAL is a key enzyme in the metabolic process of phenylpropane, which is related to the synthesis of some antibacterial substances, like many secondary phenolic compounds, lignin and flavonoid phytochemicals [22]. MDA is one of the most important products of membrane lipid peroxidation, which can measure the damage to the membrane system and plant stress resistance indirectly [23]. After JDS treatment, the activities of POD, SOD, CAT and PAL have a sharply improvement, in other hand, the content of MDA has a relative reduction. These results demonstrate that cucumber treated with JDS can effectively induce the activities of defense-related enzyme improvement, like POD, SOD, CAT, PAL, removal the damage of plant caused by increased levels of reactive oxygen due to leaf spot botrytis stresses due to *C. cassiicola* stresses, meanwhile inhibit the process of membrane lipid peroxidation in blades, to improve the resistance of cucumber seedlings to *C. cassiicola*.

Above all, under *C. cassiicola* stresses, JDS can effectively improve the content of photosynthetic pigment and how well it is growing, reduce the degradation of Chl, strengthen the occurrence of various metabolic reactions in plants, promote the enhancement of protected enzymes activities, repair enzyme protect system in cucumber leaves, reduce the level of reactive oxygen, inhibit the process of membrane lipid peroxidation in blades. In addition, it was found that the effect of JDS inducing on TLS resistance tended to weaken as the period of induction was extended, which is consistence with Sequeira [24] and Chen [25] who showed that induced disease resistance in plants has the disadvantage of being time-sensitive. Previous studies [26-28] point out that the process of inducers inducing disease resistance in plants may have some influences on cucumber Chl fluorescence parameters, photosynthetic properties and the expression of disease resistance-related protein genes, so it is necessary to experiment with the above possibilities for the next step, to clarify the resistance-inducing mechanism of the JDS induced from additional perspectives.

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COMPETING INTERESTS

Declaration of competing interest should be placed here. All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If no such declaration has been made by the authors, SDI reserves to assume and write this sentence: "Authors have declared that no competing interests exist.".

AUTHORS' CONTRIBUTIONS

Authors may use the following wordings for this section: "'Author A' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author B' and 'Author C' managed the analyses of the study. 'Author C' managed the literature searches…… All authors read and approved the final manuscript."

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