Significant effects of two pesticides on the bacteriostatic activity and antioxidant ability of green tea polyphenols

Jin Zhang*, Meng-ting Tao, Zi-yan Huang, Gui-yun Hong, Shu-guang Zhu

College of Environment and Energy Engineering, Anhui Jianzhu University, Hefei 230601, PR China

Corresponding author: Prof. Jin Zhang

College of Environment and Energy Engineering
Anhui Jianzhu University
292 Ziyun Road
Hefei 230601
P. R. China

Tell: 18019580589

E-mail address: ginnzy@163.com (J. Zhang).
Significant effects of two pesticides on the bacteriostatic activity and antioxidant ability of green tea polyphenols

Jin Zhang*, Meng-ting Tao, Zi-yan Huang, Gui-yun Hong, Shu-guang Zhu

College of Environment and Energy Engineering, Anhui Jianzhu University, Hefei 230601, PR China

Abstract

Background: Green tea polyphenols (GTPs) have good bacteriostatic activity and antioxidant capacity, yet pesticide pollutants in tea may affect their functionality. This study aims to explore the effects of pesticide pollutants on the bacteriostasis and antioxidant ability of GTPs.

Results: The bacteriostatic activity of GTPs and two pesticides (acetamiprid (ACE), diquat dibromide (DIQ)) shows some certain time characteristics. Two pesticides can affect the bacteriostatic activity of GTPs. The bacteriostatic activity of GTPs is enhanced or weakened by the two pesticides with time lengthening, i.e. time-dependent synergism or antagonism. The bacteriostatic mechanisms of the three substances and their mixtures is produced by affecting cell morphology or destroying cell structure, and the long-term antagonism of the three substances is may due to the competition of action site. In addition, the two pesticides can greatly reduce the antioxidant capacity of GTPs. ACE reduces the free radical scavenging ability of GTPs by 14%~24% and DIQ reduces the free radical scavenging ability of GTPs by 39%~63% at the experimental concentration ratios.
Conclusions: Two pesticides has significant effects on the bacteriostatic activity and antioxidant ability of GTPs.

Key words: Green tea polyphenols; Pesticides; Bacteriostatic activity; Mechanism; Antioxidant capacity

Background

Tea has become a necessity for many people. As we all known, green tea polyphenols (GTPs) accounting for 15%~30% of the dry weight of tea are the general name of polyphenols with catechin as the main component in tea, and have good bacteriostatic activity [1,2]. Ben Lagha et al. [3] pointed out that GTPs can produce bacteriostatic toxicity to Fusobacterium nucleatum by damaging cell membrane. Cho et al [4] found that GTPs can affect the cell morphology and structure of some oral bacteria.

In addition, GTPs have strong antioxidant capacity apart from bacteriostatic activity [5-7]. Therefore, a proper amount of GTPs can not only delay the aging of the human body, but also can protect the genetic material DNA of the human body from being damaged by free radicals in the process of copying [8]. This means that the loss of antioxidant capacity of GTPs will lead to the loss of its health efficacy.

However, many pesticides are used in tea to promote production. Thus, pesticides have become common residual pollutants in tea. Yi et al. [9] tested 390 tea samples on the market, and found that 52% of the samples had pesticide residue problems. Kobayash et al. [10] investigated the pesticide residues of 116 imported tea samples in Tokyo market, and found that the detection rate of pesticides in
unfermented tea was 90%. Therefore, Pesticides probably coexist with tea functional components, GTPs. Then, whether the residual pesticide pollutants in tea will affect GTPs’ bacteriostasis activity and antioxidant capacity is a matter of concern. Therefore, this study aims to explore the effects of pesticide pollutants on the bacteriostasis and antioxidant ability of GTPs. To do so, two commonly used pesticides (acetamiprid (ACE), diquat dibromide (DIQ)) in tea were selected as research objects. A freshwater luminous bacteria *Vibrio qinghaiensis* sp.-Q67 (Q67) which is very sensitive to toxic substances was selected as test organisms. The bacteriostatic ability data of GTPs, two pesticides and their binary mixtures to Q67 were determined by using time-dependent microplate toxicity analysis method (t-MTA) [11-14]. The binary mixture system of GTPs and two pesticides was constructed by using direct equipartition ray design method (EquRay) [15]. And the concentration addition model (CA) with 95% observation-based confidence interval (OCI) was used to evaluate the interaction action between pesticides and GTPs [16-20]. The bacteriostatic mechanism of the three substances and their mixtures to Q67 was preliminarily determined by observing the cellular morphology [4,21]. Besides, free radical scavenging ability of binary mixture rays was determined by the method of salicylic acid [22]. The results would provide a data reference for healthy drinking of tea.

**Materials and methods**

**Chemicals**

GTPs (Green tea polyphenols) were purchased from Shanghai Yuanye
Biotechnology Co., Ltd (Shanghai, China). Two pesticides, acetamiprid (ACE) and diquat dibromide (DIQ), were purchased from the national pesticide quality supervision and testing center of Shenyang chemical research institute (Shenyang, China). All the reagents were of analytical grade and used as received without further purification. The physical and chemical properties of the three reagents, the concentration of the stock solution, and the dilution factor \((f)\) are listed in Table 1. The storage solution was prepared with Milli-Q water and stored at 4 °C.

(Table 1 around here)

**Bacterial culture**

The freeze-dried luminescent bacterium *Vibrio qinghaiensis* sp.-Q67 (Q67) was purchased from Beijing Hamamatsu Corp., Ltd. (Beijing, China). The preparation of the culture medium and culture process of Q67 are detailed in the literature [12].

**Determination of bacteriostatic activity**

The bacteriostatic data of GTPs, two pesticides and their binary mixtures to Q67 were obtained by t-MTA. Using 96 microporous plate as experimental carrier, the relative luminous unit (RLU) of each hole were measured at 2 h, 4 h, 8 h and 12 h, respectively, and the luminous inhibition toxicity of single components and its mixtures to Q67 were calculated by Eqn(1) to reflect the bacteriostatic activity of the pollutants to Q67 at each time point. The design of the microplate is detailed in the literature [11,12].

\[
x\% = \frac{I_0 - I}{I_0} \times 100\%
\]

where \(I_0\) is the average RLU of blank control group, \(I\) is the average RLU at each
concentration gradient.

**Experimental design of mixtures**

Binary mixture system of GTPs and two pesticides was constructed by using direct equipartition ray design method (EquRay) [15]. Each mixture system contains five rays with different concentration ratios \( p_i \), and each ray was diluted to 12 fixed specific concentration points according to the dilution factor obtained by the pre-experiment [12]. The \( p_i \)s of each component of binary mixtures are shown in Table 2.

Concentration-effect curve fitting

The bacteriostatic data obtained by t-MTA were fitted by Logit (Eqn(2)) or Weibull (Eqn(3)) functions. The fitting and the calculation of 95% observation-based confidence interval (OCI) were completed by APTox software [23]. The two function formulas are as follows:

\[
E = \frac{1}{1 + \exp(-\alpha - \beta \cdot \log_{10}(c)))} \quad (2)
\]

\[
E = 1 - \exp(-\exp(\alpha + \beta \cdot \log_{10}(c))) \quad (3)
\]

where \( E \) represents the effect \((0 \leq E \leq 1)\), \( c \) represents the concentration of a single compound or mixture, \( \alpha \) and \( \beta \) represent model parameters.

**Effects of two pesticides on bacteriostatic activity of GTPs**

The interaction between GTPs and two pesticides was evaluated by using the relatively conservative CA model [16-20,24]. The calculation was completed by APTox software [23]. The function is as follows:
\[
\sum_{i=1}^{n} \frac{c_i}{EC_{x,i}} = 1
\]

where \( n \) is the number of mixture components, \( EC_{x,i} \) the concentration of the \( i \)th component that provokes \( x\% \) effect when applied individually, and \( c_i \) the concentration of the \( i \)th component in the mixture.

**Bacteriostatic mechanism to Q67**

In order to investigate the bacteriostatic mechanism of GTPs, two pesticides and their binary mixtures on Q67, the cell morphology of Q67 exposed to GTPs, two pesticides and their binary mixtures at the concentration of \( EC_{80} \) for 12 h was measured. Firstly, Q67 bacterial suspension of logarithmic growth period was placed in conical flask. Then, GTPs, two pesticides and their binary mixtures were added to the conical flask, respectively, so that the concentration of drugs in the suspension was equal to the \( EC_{80} \) of GTPs, two pesticides and their binary mixtures at 12 h. Lastly, the conical flask was cultured in a constant temperature incubator at \( 22 \pm 1 \) °C for 12 h. After 12 h, the cell morphology of Q67 was observed by scanning electron microscope. The specific steps for the preparation of electron microscope samples refer to the relevant literature [4,21].

**The effects of two pesticides on the antioxidative ability of GTPs**

The free radical scavenging ability of GTPs and two pesticide binary mixtures under different \( p{s}s \) (experimental group) were determined by the method of salicylic acid [22]. Then, replace pesticides in binary mixtures with equal volume Milli-Q water as control (blank group) to determine the free radical scavenging ability of GTPs. The greater the difference between the free radical scavenging ability of the
experimental group and the control group is, the greater the effects of pesticides on the antioxidant capacity of GTPs.

**Results and discussion**

**Bacteriostatic activity of individual components to Q67**

The nonlinear least square method was used to fit the concentration-response data of GTPs and two pesticides to Q67 at 0.25 h, 2 h, 4 h, 8 h and 12 h. The results show that the Weibull function can effectively characterize the concentration-response relationship of GTPs, ACE and DIQ ($R>0.9$, $RMSE<0.1$). The specific fitting results and relevant statistic parameters are shown in Table 1. The time-concentration-response curves (t-CRCs) of single components are shown in Fig.1.

From Fig.1, the time characteristics of the bacteriostatic activity of GTPs and two pesticides to Q67 are different. The bacteriostatic activity of GTPs increases with the prolongation of exposure time within 0.25 ~ 12 h, while the bacteriostatic activity of DIQ decreases within 0.25 ~ 2 h, but increases within 2 ~ 12 h. The bacteriostatic activity of ACE to Q67 is not affected by exposure time, and its t-CRCs almost coincide at five exposure time points. Combined with Table 1, taking pEC$_{50}$ ($-\lg EC_{50}$) as the bacteriostatic activity index, the bacteriostatic activity order is as follows at 0.25 h and 2 h: ACE ($pEC_{50}=3.44 \sim 3.47$) > GTPs ($pEC_{50}=3.17 \sim 3.26$) > DIQ ($pEC_{50}=3.81 \sim 3.85$). The bacteriostatic activity order is as follows at 4 h, 8 h and 12 h: DIQ ($pEC_{50}=3.81 \sim 6.79$) > GTPs ($pEC_{50}=3.61 \sim 4.14$) > ACE ($pEC_{50}=3.55 \sim 3.60$).

In conclusion, the antibacterial effect of GTPs and two pesticides on Q67 varies
with the exposure time [25]. And it is noted that in addition to the good bacteriostatic activity of GTPs, two pesticides, as toxic substances, also have inhibitory toxicity to bacteria due they are pesticides with high toxicity to nontarget organisms [26,27].

(Fig.1 around here)

Effects of two pesticides on bacteriostatic activity of GTPs

Weibull function can also effectively characterize the concentration-response relationship of binary mixture rays of GTPs and two pesticides (R>0.9, RMSE<0.1).

The fitting results and relevant statistic parameters are shown in Table S1. The t-CRCs of binary mixture rays are shown in Figure S1. The results of the CA prediction are not always in agreement with the experimental results, and when the CA prediction curve is higher or lower than the OCI, there is an interaction between the components of the mixture [16-20,28]. The experimental observations of each mixture ray with obvious synergism at different time points and its 95% OCI, fitting curves and CA prediction results are shown in Fig. 2 and Fig. 3. (Other results are drawn in Figure S2).

(Fig.2 around here)

(Fig.3 around here)

It can be seen from Fig. 2 and Fig. 3 that the five rays of the GTPs-ACE binary mixture system show synergism and additive action in exposure times of 0.25 h and 2 h, and the CA prediction line is located below or between the OCI. However, with the prolongation of exposure time, five mixture rays all show antagonism, and the CA prediction line is located above the OCIs. The five mixture rays of GTPs-DIQ binary
mixture system show synergism when the exposure time is 0.25 h, but with the prolongation of exposure time, the toxicological interaction changes from synergism to antagonism. Therefore, the toxicological interaction of the two groups of binary mixtures tends to antagonism with the prolongation of exposure time.

So, from the results of toxicity interaction analysis, it can be seen that ACE and DIQ can affect the bacteriostatic activity of GTPs, and the influence mode differs in different exposure time. The short-term synergism of the mixture system will strengthen the bacteriostatic ability of GTPs, while the long-term antagonism of the mixture system will lead to the decrease of bacteriostatic ability of GTPs. Therefore, pesticide pollutants will affect the functionality of GTPs from the point of view of bacteriostatic activity.

The bacteriostatic mechanism to Q67

The cell morphology of Q67 exposed to the EC₈₀ of GTPs, two pesticides and their representative rays for 12h is shown in Fig. 4. From Fig. 4, the morphology of Q67 cells is changed compared with the control group. GTPs and DIQ make Q67 cells bond with each other and destroyed the membrane structure of bacteria, which make the contents of Q67 flowed out. While ACE prolonged Q67 cells and ruptured Q67 cells. The rays with the greatest long-term bacteriostatic activity in two binary mixture systems were selected to observe their effects on morphology of Q67 cells. It is found that mixtures also damaged the morphology of Q67 cells. Besides, it is found that the amount of bacteria in the experimental group is lower than that in the control group after centrifugation. This suggests that GTPs, two pesticides and their mixtures
can inhibit the reproduction of bacteria. Cho et al [4] also found that tea polyphenols could destroy the cell structure of the bacteria. Therefore, the three substances may have similar antibacterial mechanisms, and the long-term antagonism of the three substances is due to competition of action site [29].

(Fig. 4 around here)

**Effects of two pesticides on the antioxidative ability of GTPs**

In order to characterize the effects of two pesticides on the antioxidative ability of GTPs, the free radical scavenging ability of each binary mixture ray was determined by salicylic acid method. The results are shown in Fig. 5. As can be seen from Fig. 5, the free radical scavenging ability of each binary mixture ray is lower than that of the control group. ACE reduces the free radical scavenging ability of GTPs by 14%~24% and DIQ reduces the free radical scavenging ability of GTPs by 39%~63% at the experimental concentration ratios. This indicates that pesticide pollutants can damage the antioxidant capacity of GTPs, and the extent of damage is DIQ > ACE. Therefore, antioxidant capacity of GTPs will be greatly affected by residual pesticides, which will affect the quality of the tea by weakening GTPs’ functionality.

(Fig.5 around here)

**Conclusion**

GTPs and two pesticides all have antibacterial activity, and its bacteriostatic activity shows some certain time characteristics. Both ACE and DIQ can affect the bacteriostatic activity of GTPs. The bacteriostatic activity of GTPs will be enhanced
when the two pesticides and GTPs coexist for a short time. However, the bacteriostatic activity of GTPs will be weakened when the two pesticides and GTPs coexist for a longer time. That is to say, mixtures of the two pesticides and GTPs exhibit time-dependent synergism or antagonism. The bacteriostatic mechanism of GTPs, two pesticides and their mixtures on Q67 is produced by affecting cell morphology or destroying cell structure. Therefore, GTPs and two pesticides may have similar antibacterial mechanisms, and the long-term antagonism of the three substances is due to the competition of action site. In addition, the two pesticides can greatly reduce the antioxidant capacity of GTPs, and the extent of damage is DIQ > ACE.

**Abbreviations**

GTPs: Green tea polyphenols; ACE: Acetamiprid; DIQ:Diquat dibromide; Q67: *Vibrio qinghaiensis* sp.-Q67; t-MTA: Time-dependent microplate toxicity analysis method; EquRay: Direct equipartition ray design method; CA: Concentration addition model; OCI: 95% observation-based confidence interval; RLU: Relative luminous unit; t-CRCs: Time-concentration-response curves.

**Declarations**

**Acknowledgments**

We would like to thank financial support from the National Natural Science Foundation of China, Natural Science Foundation and Technology Project of Anhui Province in China.
Authors’ contributions

The corresponding author JZ is responsible for ensuring that the descriptions are accurate and agreed by all authors. The author MT is responsible for investigation and writing -original draft, and ZH is responsible for formal analysis. GH is responsible for resources, and SZ is responsible for supervision.

Funding

The authors are especially grateful to the National Natural Science Foundation of China (No. 21677001), Natural Science Foundation of Anhui Province, China (No. 1708085MB50) and Technology Project of Anhui Province (Grant No. 17030801028) for their financial support.

Availability of data and materials

All data supporting the conclusions of this article are included within the article and one additional file.

Author information

Affiliations

College of Environment and Energy Engineering, Anhui Jianzhu University, Hefei 230601, PR China

Corresponding author

Correspondence to Jin Zhang.

Ethics approval and consent to participate

Not applicable.

Consent for publication
Competing interests
The authors declare that they have no competing interests.

References
1. Sakanaka S, Raj JL, Taniguchi M (2000) Antimicrobial effects of green tea polyphenols on thermophilic spore-forming bacteria. J Biosci Bioeng 90: 81-85. https://doi.org/10.1016/S1389-1723(00)80038-9
2. Bansal S, Syan N, Mathur P, Choudhary S (2012) Pharmacological profile of green tea and its polyphenols: a review. Med Chem Res 21: 3347-3360. https://doi.org/10.1007/s00044-011-9800-4
3. Ben Lagha A, Haas B, Grenier D (2017) Tea polyphenols inhibit the growth and virulence properties of Fusobacterium nucleatum. Sci Rep 7: 44815. https://doi.org/10.1038/srep44815
4. Cho YS, Oh JJ, Oh KH (2010) Antimicrobial activity and biofilm formation inhibition of green tea polyphenols on human teeth. Biotechnol Bioreoc E 15: 359-364. https://doi.org/10.1007/s12257-009-0195-8
5. Frei B, Higdon JV (2003) Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr 133: 3275S-3284S. https://doi.org/10.1046/j.1365-277X.2003.00466.x
6. Coentrão PA, Teixeira VL, Netto AD (2011) Antioxidant activity of polyphenols from green and toasted mate tea. Nat Prod Commun 6: 651-656.
7. Wang Y (2007) The anti-oxidation and anti-microbial activities of tea polyphenols and its increased reagents. J Biol 24: 54-56. https://doi.org/10.1016/S1872-2075(07)60055-7

8. Lodovici M, Casalini C, Filippo CD, Copeland E, Dolara P (2000) Inhibition of 1,2 - dimethylhydrazine - induced oxidative DNA damage in rat colon mucosa by black tea complex polyphenols. Food Chem Toxicol 38: 1085-1088. https://doi.org/10.1016/S0278-6915(00)00109-5

9. Yi J, Yang M, Nie Y, Tu FQ, Kang CX, Zhou Z, Zhu JX, He R, Hu XJ (2019) Monitoring and risk assessment of 30 kinds of pesticide residues in tea samples. Modern Food Science and Technology 35: 250-257. https://doi.org/10.13982/j.mfst.1673-9078.2019.4.034 (in Chinese)

10. Kobayash M, Ohtsuka K, Tamura Y, Tomizawa S, Kinoshita T, Kamijo K, Iwakoshi K, Sato C, Nagayama T, Takano I (2013) Survey of Pesticide Residues in Imported Tea (1992.4 - 2010.3). J Food Hyg Soc Jpn 54: 224-231. https://doi.org/10.13982/10.3358/shokueishi.54.224

11. Zhang J, Ding TT, Dong XQ, Bian ZQ (2018) Time-dependent and Pb-dependent antagonism and synergism towards Vibrio qinghaiensis sp.-Q67 within heavy metal mixtures. RSC Adv 8: 26089-26089. https://doi.org/10.1039/C8RA04191A

12. Zhang J, Liu SS, Dong XQ, Chen M (2015) Predictability of the time-dependent toxicities of aminoglycoside antibiotic mixtures to Vibrio qinghaiensis sp.-Q67.
13. Xu Y, Li K, Wang Z, Liu SS (2020) The weak magnetic field (WMF) enhances the stimulation of polymyxin B sulfate (POL) on Vibrio qinghaiensis sp.-Q67. Environ Sci Eur 32: 11. https://doi.org/10.1186/s12302-020-0294-x

14. Feng L, Liu SS, Li K, Tang, HX, Liu HL (2017) The time-dependent synergism of the six-component mixtures of substituted phenols, pesticides and ionic liquids to Caenorhabditis elegans. J Hazard Mater 327: 11-17. https://doi.org/10.1016/j.jhazmat.2016.12.031

15. Dou RN, Liu SS, Mo LY, Liu HL, Deng FC (2010) A novel direct equipartition ray design (equray) procedure for toxicity interaction between ionic liquid and dichlorvos. Environ. Sci Pollut R 18: 734-742. https://doi.org/10.1007/s11356-010-0419-7

16. Howard GJ, Webster TF (2009) Generalized concentration addition: a method for examining mixtures containing partial agonists. J Theor Biol 259: 469-477. https://doi.org/10.1016/j.jtbi.2009.03.030

17. Backhaus T, Faust M, Scholze M, Gramatica P, Vighi M, Grimme LH (2004) Joint algal toxicity of phenylurea herbicides is equally predictable by concentration addition and independent action. Environ Toxicol Chem 23: 258-264. https://doi.org/10.1897/02-497

18. Iwasaki Y, Gauthier P (2016) Concentration addition and response addition to analyze mixture toxicity: Is it worth testing? Environ Toxicol Chem 35: 526-527. https://doi.org/10.1002/etc.3263
19. Cedergreen N, Sorensen H, Svendsen C (2012) Can the joint effect of ternary mixtures be predicted from binary mixture toxicity results? Sci Total Environ 427: 229-237. https://doi.org/10.1016/j.scitotenv.2012.03.086

20. Zhang J, Liu SS, Dou RN, Liu HL, Zhang J (2011) Evaluation on the toxicity of ionic liquid mixture with antagonism and synergism to Vibrio qinghaiensis sp.-Q67. Chemosphere 82: 1024-1029. https://doi.org/10.1016/j.chemosphere.2010.10.063

21. Ng LK, Sherburne R, Taylor DE, Stiles ME (1985) Morphological forms and viability of Campylobacter species studied by electron microscopy. J Bacteriol 164: 338-343. https://doi.org/0021-9193/85/100338-06$02.00/0

22. Li NW, Liu CH, Huang K (2011) Extraction and free radical scavenging activity of total flavonoids from pumpkin seedlings. Food Sci 32: 58-60. https://doi.org/10.3724/SP.J.1011.2011.00211 (in Chinese)

23. Liu SS, Zhang J, Zhang YH, Tan LT (2012) APTox: Assessment and prediction on toxicity of chemical mixtures. Acta Chimica Sinica 70: 1511-1517. https://doi.org/10.6023/A12050175 (in Chinese)

24. Qu R, Liu SS, Chen F, Li K (2016) Complex toxicological interaction between ionic liquid and pesticide to Vibrio qinghaiensis sp.-Q67. RSC Adv 6: 21012-21018. https://doi.org/10.1039/C5RA27096K

25. Baas J, Jager T, Kooijman B (2010) Understanding toxicity as processes in time. Sci Total Environ 408: 3735-3739. https://doi.org/10.1016/j.scitotenv.2009.10.066.
26. Ray RC (1983) Toxicity of the pesticides hexachlorocyclohexane and benomyl to nitrifying bacteria in flooded autoclaved soil and in culture media. Environ Pollut 32: 147-155. https://doi.org/10.1016/0143-1471(83)90047-8

27. G. Bermudez-Humaran L, Langella P (2012) Importance of commensal and probiotic bacteria in human health. Curr Inorg Chem 8: 248-253. https://doi.org/10.2174/157339512800671994

28. Fan Y, Liu SS, Qu R, Li K, Liu HL (2017) Polymyxin B sulfate inducing time-dependent antagonism of the mixtures of pesticide, ionic liquids, and antibiotics to *Vibrio qinghaiensis* sp.-Q67. RSC Adv 7: 6080-6088. https://doi.org/10.1039/C6RA25843C

29. Groten JP, Feron VJ, Jürgen Sühnel (2001) Toxicology of simple and complex mixtures. Trends Pharmacol Sci 22: 316-322. https://doi.org/10.1016/S0165-6147(00)01720-X