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# Inorganic salts and salicylic acid for the control of *Rhizopus stolonifer* on plum

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## Abstract

Plum (*Prunus domestica* L.) is an important stone fruit grown in different parts of world and has high nutritive as well as medicinal values. Many diseases attack on plum, among these Rhizopus rot, caused by *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill is a main postharvest disease of economic worth worldwide which managed by the use of several conventional chemicals but due to development of resistance and factor of exposure risks, fungicide residues and human health hazards have given a push for obtaining alternatives strategies. Therefore, in the present study two different alternative management strategies such as use of inorganic salts and salicylic acid were evaluated to check their fungitoxic effect to control fungus development using poison food technique. Results showed that both inorganic salts and salicylic acid found to be most effective to inhibit the fungal mycelial growth at all tested concentrations. Among inorganic salts, potassium bicarbonate and sodium carbonate were found most effective to control mycelial growth as compared to potassium carbonate and sodium bicarbonate which proved less effective in both conditions respectively. Application of SA at 0.025%, 0.05%, 0.075% and 0.1% inhabit fungal development of 30.28%, 63.64%,100% and up to 100% respectively. Inhibition percentage was gradually increased with the increasing of incorporated concentrations. Therefore, the present study showed that inorganic salts and salicylic acid can be best alternatives to chemicals beings less health hazardous to human being and environment.

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## Introduction

Plum (*Prunus domestica* L.) is a significant stone fruit of Pakistan (Shahzad *et al.,* 2013). Plum has high nutritional worth as it contains vitamin A, B, C and K, calcium, iron, dietary fiber, antioxidants, sugars and potassium (Ertekin *et al*., 2006; Rao *et al*., 2009; Prajapati *et al*., 2012).

Plum fruits are prone to different fungal, bacterial, viral and nematode diseases. Among fungal diseases Rhizopus rot caused by *R. stolonifer* is most caustic post-harvest disease of stone fruits worldwide (Kwon *et al*., 2001). It caused black sphere-shaped structure and formed white whiskery mold on surface of fruit and can damage whole fruit within approximately two days. It can also produced thick walled spores under unfavorable conditions. When the situation turns into normal these thick walled spores rupture and cause of disease development (Gonçalves *et al*., 2010).

Currently, synthetic chemicals have been frequently used to control post-harvest diseases in different ways like aqueous solution, fumigation and dipping the fruits in soak tanks. Chemical fungicides are unsafe for human health due to health hazardous and also have unfavorable effect on environment (Singh *et al*., 2009; Calvo *et al*., 2007). In developed countries, many chemical fungicides such as guazatine for controlling sour rot of citrus are banned for export (Cunningham and Taverner, 2007). Therefore, possible alternatives used which are more efficient, inexpensive and eco-friendly against postharvest diseases. An environmental friendly alternative is the use of elicitors that may induce resistance against the pathogens without disturbing environment. Actually induce resistance results in activation of plant defense system by non-toxic chemicals (Olivieri *et al*., 2009). Salicylic acid (SA) is plant hormone present in plant tissues and has no aroma (Shakirova, 2007; Hayat and Ahmad, 2007). It's primarily function is to activate plant defense and induces resistance against postharvest pathogens in fruits (Hussain *et al*., 2014). Therefore, different chemical elicitors (in organic salts and salicylic acid) used to control the incidence of rhizophus rot on plum (Abdelhamid *et al*., 1985; Ziv and Zitter, 1992). So, the current study was planned to assess different chemical elicitors alone and their probable combinations on plum fruits under *in vitro* as well as in field conditions for controlling *R. stolonifer* causal agent of Rhizopus rot in Plum.

## Materials and methods

The present research work was done in the laboratory of plant pathology, College of Agriculture, Sargodha to assess the efficiency of different chemical elicitors against postharvest disease of plum viz. Rhizophus rot under *in vitro* and on harvested fruits. Two types of elicitors were used i.e inorganic salts and salicylic acid.

Diseased fruit having typical symptoms were collected from the markets, of Sargodha. The infected fruits washed carefully with the distilled water to remove waste/debris and with 1% sodium hypochlorite for surface sterilization. Then small pieces were transferred to the petri plates containing potato dextrose agar medium. Plates were sealed with parafilm and incubated at 25ºC for one week. Fungal mycelial growth was observed after four days of incubation. Pathogenicity was done to confirm the association with host according to Koch's postulates. Pure cultures of pathogen were preserved at 4Cº on PDA medium/slants for further experiments.

### *Inoculum preparation*

Pure culture of *R. stolonifer* was prepared on petri plates containing PDA media. Ten milliliter sterilized double distilled water was added into petri plates containing seven days old culture and resulted suspension was filtered to remove mycelial fragments of the target pathogen.

### *Pathogenicity*

Healthy fruits of plum were taken from the fruit market, sterilized and inoculated with prepared suspension of pathogen with sprayer. The inoculated fruits were kept in growth chamber for seven days and examined regularly for symptoms development. After the symptoms appeared on fruits, re-isolation was done and compared with pure culture to confirm the pathogen.

*Evaluation of elicitors*

*Chemical elicitors*

Inorganic salts like sodium carbonate, sodium bicarbonate, potassium carbonate and potassium bicarbonate were used at 1%, 2%, 3%, and 4% concentration with check treatment (control) through poisoned food technique. The chemical names and their formula were presented (Table 1).

*In vitro evaluation of inorganic salts*

The efficiency of salts i.e. sodium carbonate ($Na_2CO_3$), sodium bicarbonate ($NaHCO_3$), potassium carbonate ($K_2CO_3$) and potassium bicarbonate ($KHCO_3$) against *R. stolonifer* were evaluated using food poison technique. Salts of all concentrations (1%, 2%, 3% and 4%) were amended into molted potato dextrose agar (PDA) medium at the rate of 1:4 (v/v) and poured into Petri plates. A 5mm agar disc from pure cultures of fungus was placed at the center of each plate and incubated at 24°C. PDA medium without salts served as control.

The experiment was included three replications with three petri plates for each treatment. Mycelial growth of fungus colony was measured after 5 days of colony growth and percent inhibition efficiency for all inorganic salts was calculated by using formula (McKinney, 1927).

Inhibition Percentage (IP) = Control – Treatment × 100Control

*Evaluation of salts on harvested plum fruits*

The plum fruits were disinfected with 70% ethanol for 2 minutes and followed by drying under sterile condition at room temperature. After that fruits were dipped in different concentration (1%, 2%, 3% and 4%) of salts for 30 minutes and allowed to dry at room temperature. The twenty five micro liters of $10^5$ conidia/mL of the pathogen were inoculated into wounded fruits and then fruits were placed in plastic bags and stored at 24°C for 3 days. There were 3 fruits for each treatment and each treatment was replicated for 3 times.

*Use of Salicylic Acid*

*In vitro experiment*

The efficacy of SA on the radial growth of *R. stolonifer* was assessed according to methodology explained by Yao and Tian (2005). SA was primarily dissolved in 1% ethanol and then subsequent required concentrations were prepared in distilled water. Four concentrations (0.025%, 0.05%, 0.075% and 0.1%) of SA was prepared and amended into molted PDA medium. When PDA medium solidified, 5mm discs of *R. stolonifer* was put into middle of each Petri dish and incubated at 24±1°C. The data of mycelium growth was recorded at 48, 72, 96 and 120h .The treatment without amendment was allocated as control. After incubation period, mycelial growth of fungus colony was measured after 5 day and percent inhibition efficiency calculated with formula of McKinney, (1927).

*In vivo experiment*

Fruits: Fresh and healthy plum fruits of the same size and maturity without any fungicide treatment for present study were collected from greenhouses. All the fruits were of same size and maturity. Selected fruits were cleaned with 1% solution of sodium hypochlorite for 5 mint followed by washing with distilled water and subject to natural air drying at room temperature for further studies.

Preparation of spore suspension: The suspension of spore was prepared by adding 10 ml distilled water into pure colony of 10 days old culture and glass rod was used to detached conidia from mycelium. The conc. was adjusted to 1 × $10^5$ spores/ml through hemocytometer. The conidial suspension was set at the rate of 0.05% (v/v) in tween 20.

Surface sterilized fruits were dipped into SA solution (0.025%, 0.05%, 0.075% and 0.1%) for 30 minutes and control fruits were in sterilized water. The inoculation was done by inserting 30µl spore suspension of each pathogen into wounded (0.5 mm in diameter by 5 mm in depth) fruits. The data was recorded at 48, 72 and 96, 120h on regular basis.

*Statistical analysis*

All the experiments were repetitive three times for reducing experimental errors. Analysis of variance of collected data was analyzed under 2-factor factorial experimental design and means were compared by latin square difference (LSD) using R-software.

**Results**

*In organic salts*

Results showed that at 1% and 2% conc. none of in organic salt inhabited fungal mycelium growth effectively in both conditions and can't suppress fungal development (Tables 1-2). Somehow under lab conditions salts e.g potassium bicarbonate (78.96%) and sodium carbonate (77.31%) when applied at 4% showed significant results and inhabited fungal development while potassium carbonate (74.66%) and sodium bicarbonate (72.29%) found to be less effective (Fig 1). The results were found as sodium carbonate, sodium bicarbonate, potassium carbonate and potassium bicarbonate inhabited fungal growth of 78.76%, 74.1%, 76.33% and 80.33% respectively when applied on harvested fruits (Fig. 2). It can be concluded from above results that salt showing good inhibition percentage in both lab and on harvested fruits when applied.

**Table 1.** Effects of different concentrations of salts on Rhizopus rot of plum caused by *R. stolonifer in* lab conditions.

| Treatments | Time Interval | | | | |
|---|---|---|---|---|---|
| | Dose | 48h | 72h | 96h | 120h |
| Sodium carbonate (SC) | 1% | 13.13±0.65b | 28.20±0.29c | 44.66±0.80c | 67.53±1.10c |
| | 2% | 8.82±0.38bc | 18.98±0.24c | 30.13±0.59c | 45±0.77c |
| | 3% | 5.93±0.25ab | 12.69±0.13b | 20.20±0.39c | 30.45±0.51c |
| | 4% | 3.97±0.19a | 8.53±0.11 | 13.55±0.27b | 20.41±0.40c |
| Sodium bicarbonate (SBC) | 1% | 16.06±0.69a | 34.50±0.43a | 54.46±1.29a | 73.24±0.57b |
| | 2% | 10.78±0.48a | 23.11±0.30a | 36.75±0.68a | 58.11±1.05a |
| | 3% | 7.23±0.30a | 15.53±0.17a | 24.66±0.47a | 37.18±0.62a |
| | 4% | 4.86±0.25a | 10.43±0.14 | 16.39±0.35a | 24.93±0.41a |
| Potassium carbonate (PC) | 1% | 14.90±0.65a | 31.86±0.24b | 50.76±0.73b | 76.39±1.28a |
| | 2% | 9.98±0.42ab | 21.34±0.16b | 33.91±0.66b | 51.25±0.86b |
| | 3% | 6.61±0.33ab | 14.42±0.19a | 22.76±0.48b | 34.4±0.60b |
| | 4% | 4.49±0.19a | 9.73±0.18ab | 15.31±0.35a | 22.8±0.54b |
| Potassium bicarbonate (PBC) | 1% | 12.18±0.53b | 26.1±0.40d | 41.56±0.86d | 62.45±1.12d |
| | 2% | 8.13±0.40c | 17.54±0.25c | 27.88±0.56d | 41.91±0.74d |
| | 3% | 5.48±0.27b | 11.77±0.14b | 18.71±0.38c | 28.02±0.48d |
| | 4% | 3.69±1.95a | 7.94±0.09c | 12.56±0.25b | 18.93±0.33c |

The overall experimental results showed that by increasing conc. of salts and SA the fungal development gradually decreased (Figs 1-2).

*Salicylic acid*

The mycelium growth of *R. stolonifer* was totally inhibited at 0.075% and 0.1% conc. of SA in lab conditions compared with control. Fungal mycelium growth was started on second day and a clear difference was seen in growth diameter observable on third day (Table 3).

The 0.05% conc. of SA showed to be significantly effective and provided 67.17% growth inhibition of *R. stolonifer* after 5 days (Fig. 3) by inducing resistance in Plum plant.

**Table 2.** Effects of different concentrations of salts on Rhizopus rot of plum caused by *R. stolonifer in* field conditions.

| Treatments | Dose | 48h | 72h | 96h | 120h |
|---|---|---|---|---|---|
| Sodium carbonate (SC) | 1% | 4.1±0.20a | 8.8±0.10c | 13.9±0.25c | 21±0.35c |
| | 2% | 2.75±0.12a | 5.91±0.07b | 9.39±0.18c | 14.14±0.24c |
| | 3% | 1.85±0.08a | 3.95±0.04bc | 6.29±0.12b | 9.49±0.16c |
| | 4% | 1.24±0.06a | 2.66±0.03sb | 4.22±0.08bc | 6.37±0.11c |
| Sodium bicarbonate (SBC) | 1% | 5.01±0.21bc | 10.75±0.13a | 17.07±0.33a | 23±0.15b |
| | 2% | 3.36±0.15ab | 7.21±0.09a | 11.45±0.22a | 17.24±0.29a |
| | 3% | 2.25±0.09a | 4.84±0.055a | 7.68±0.14a | 11.58±0.19a |
| | 4% | 1.51±0.05a | 3.25±0.04a | 5.11±0.10a | 7.77±0.13a |
| Potassium carbonate (PC) | 1% | 4.64±0.20ab | 9.92±0.07b | 15.82±0.24b | 23.8±0.40a |
| | 2% | 3.11±0.13ab | 6.65±0.05a | 10.56±0.20b | 15.96±0.25b |
| | 3% | 2±0.11a | 4.49±0.06ab | 7.09±0.15a | 10.72±0.18b |
| | 4% | 1.4±0.06a | 3.03±0.05ab | 4.78±0.10ab | 7.1±0.17b |
| Potassium bicarbonate (PBC) | 1% | 3.79±0.16c | 8.13±0.13d | 12.95±0.25d | 19.46±0.35d |
| | 2% | 2.53±0.13b | 5.47±0.07b | 8.69±0.17d | 13±0.23d |
| | 3% | 1.71±0.08a | 3.67±0.45c | 5.83±0.11b | 8.73±0.15d |
| | 4% | 1.15±0.08a | 2.48±0.03b | 3.91±0.07c | 5.9±0.10c |

The decrease in mycelium growth was might be due to antifungal characteristics of SA. All the conc. of SA applied on harvested plum fruit by dipping method reduced lesion with significantly as compared to the control. However complete fungal development was inhabited by SA at concentrations of 0.075% and 0.1% under field conditions after inoculation at proper temperature. SA at 0.025 % concentration have highest lesion diameter. SA at different conc. i.e. 0.025%, 0.05%, 0.075% and 0.1% inhabit fungal development of 30.28%, 63.64%, 100% and up to 100% respectively (Fig. 4).

**Table 3.** Effects of different concentrations of Salicylic acid on Rhizopus rot of plum caused by *R. stolonifer* applied as food poison technique and stored at 24±1°C *in vitro* and on harvested fruits *in* field conditions.

| Treatment | Doses | Lab Conditions 48h | Lab Conditions 72h | Lab Conditions 96h | Lab Conditions 120h | Field conditions 48h | Field conditions 72h | Field conditions 96h | Field conditions 120h |
|---|---|---|---|---|---|---|---|---|---|
| R. stolonifer (Salicylic acid (SA), Time interval) | 0.025% | 15.84± 0.04b | 29.18± 0.55 | 40.5± 2.70b | 56.53± 1.84b | 5.84± 0.04a | 10.76± 0.16a | 14.98± 1a | 20.91± 0.68a |
| | 0.05% | 2.29± 0.08c | 5.86± 0.33c | 13.95± 0.15c | 29.54± 0.66c | 0b | 2.1± 0.10b | 5.12± 0.1b | 10.9± 0.27b |
| | 0.075% | 0d | 0d | 0d | 0d | 0b | 0b | 0c | 0 |
| | 1% | 0d | 0d | 0d | 0d | 0b | 0b | 0c | 0 |
| | Control | 26.71± 3.25a | 41.30± 1.33a | 67.88± 2.90a | 90± 0.00a | 6.67± 0.05a | 12.86± 0.13a | 21± 0.45a | 28.16± 0.53a |

## Discussion

Management of Rhizopus rot through the use of fungicides is not expensive method but its hazard problems left no choice for farmers to use alternative methods. Hence, as alternative to fungicides the effects of chemical elicitors (in-organic salts and salicylic acid) were evaluated for their inhibitory effect on linear growth of *R. stolonifer*, causal agent of Rhizopus rot. Results of chemical elicitors indicated that the inhibition percentage was increased by increasing the tested concentrations of chemical elicitors. All tested salts at all conc. have good effects on fungal development but potassium bicarbonate, sodium bicarbonate and salicylic acid proved to be the most effective for retarding fungal mycelia growth both in lab and field conditions. Palou *et al.* (2001) evaluated the efficacy of sodium carbonate and observed that SC inhibited the growth of pathogen up to 90% which is almost similar to our findings. Droby *et al.* (2003) confirmed that sodium bicarbonate was the most reliable in providing antifungal action when used against *Rhizopus stolonifer* and use of these salts is a useful approach to improve the efficiency of fungal antagonists used for control of post harvest disease.

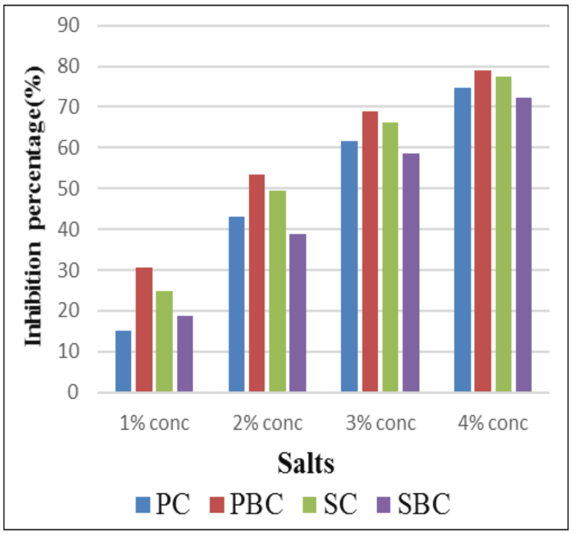


**Fig. 1.** Growth inhibition (%) of *R.Stolonifer* by different concentrations of salts *in vitro*.

Masoud (2014) conducted an experiment to check the efficacy of inorganic salts (sodium bicarbonate and potassium bicarbonate) and its results proved to be effective against many other plant fungal pathogens when used in integrated manner.

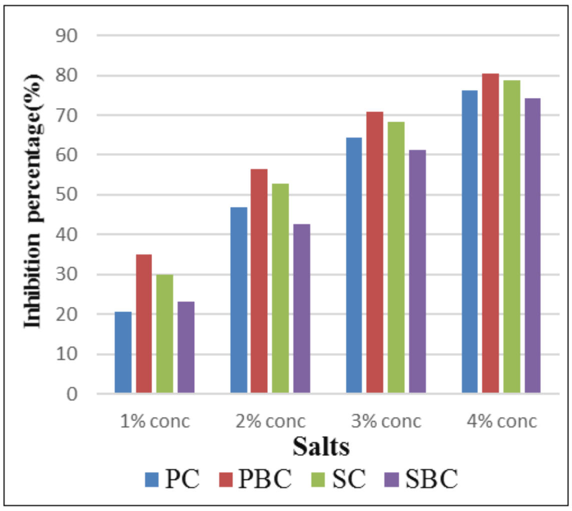


**Fig. 2.** Growth inhibition (%) of *R.Stolonifer* by different concentrations of salts on harvested fruits.

However, there are some reports which are contrary to our results as Feliziani *et al.* (2013) who reported that potassium bicarbonate are not so effective to control *R. stolonifer* but more documents are available in favor of proving the antifungal efficacy of these in-organic salts.

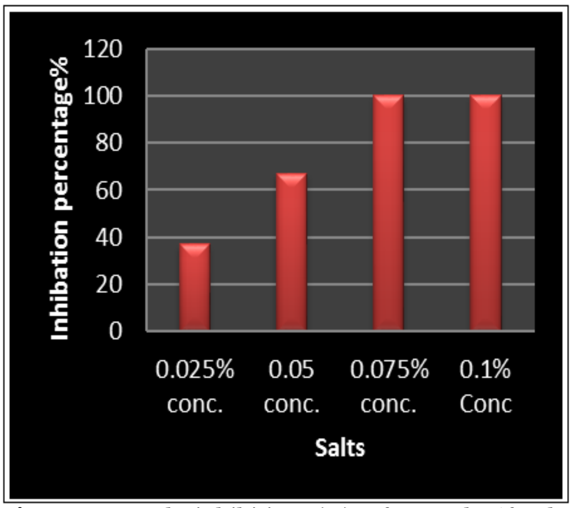


**Fig. 3.** Growth inhibition (%) of *R.Stolonifer* by different concentrations of salicylic acid *in vitro*.

Our results are to some extent also coordinated with the results obtained by Keykha *et al.* (2014); Wodnicka *et al.* (2017); War *et al.* (2011); Panahirad *et al.* (2012) who reported that salicylic acid significantly reduced linear growth of many pathogen including *Rhizopus stolonifer* in both lab as well as in field conditions. Panahirad *et al.* (2012) evaluated the efficacy of SA used against *R. stolonifer* and observed that SA significantly reduced Rhizopus growth at all concentrations which are similar to our results.

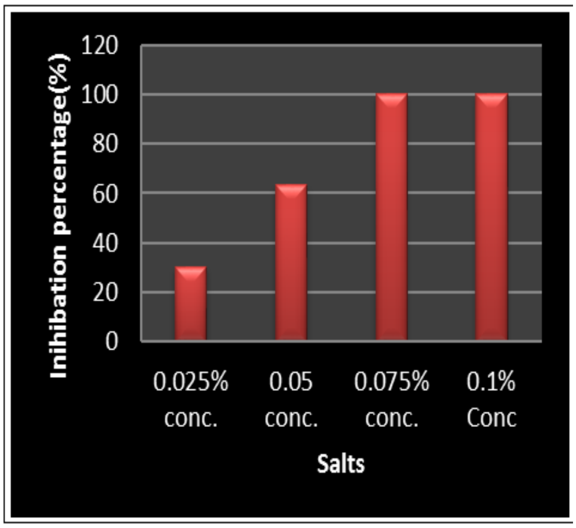


**Fig. 4.** Growth inhibition (%) of *R.Stolonifer* by different concentrations of salicylic acid on harvested fruits.

Basically It's primarily function is to activate plant defense and induces resistance against postharvest pathogens in fruits (Hussain *et al.*, 2014).

Previously fungicidal effect of SA has been reported against different postharvest pathogens more effectively like, *Penicillium digitatum, Alternaria alternata*, *P. expansum*, *Monilinia fructicola* and *P. italicum*in citrus & *Botrytis fabae* (Aldesuquy *et al.*, 2012; Wang and Li, 2008; Iqbal *et al.*, 2012). Actually chemical elicitors do not eradicate pathogens; they activate plant resistance mechanisms, one of which is to enhance the level of phenolic compounds.

Therefore, their functions not only allow us to manage plant diseases but also to boost the phenolic content of plant foodstuffs (Ruiz-García and Gómez-Plaza, 2013). Therefore, from the foregoing argument it may be accomplished that chemical elicitors could be use as effective source for induce resistance in plants against several pathogens including *R. stolonifer* for controlling Rhizopus rot.

**Conclusion**

Alternative management strategies to control this devastating disease are necessary to prevent losses caused by pathogen during post-harvest handling of fruit. Results showed that sodium carbonate and potassium bicarbonate proved to be more effective than rest of all salts while salicylic acid used at four different concentrations inhabited fungal development completely at highest concentration in both conditions.

Therefore, the increase in fruit resistance to infection of plant pathogens through the use of eco-friendly and low toxicity substances has become a more sui**table** and an effective alternative strategy for the control of plant pathogens.

**Recommendations**

The alternative strategies should be developed and integrated with other control strategies to suppress plant disease, increase yield, and protect environment and biological resources in sustainable agricultural system.

**Author's Contribution**

Dr. Muhammad Usman Ghazanfar conceived the idea and facilitated, guided and supervised the experiment. Mr. Mustansar Abbas planned and executed the experiment and also noted the results. Mr. Waqas Raza reviewed and finalized the manuscript.

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**Conflict of Interest**

"The author(s) declare(s) that there is no conflict of interests regarding the publication of this article".

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