Biological Forum – An International Journal **15(10): 365-370(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Evaluation of Plant Extracts against *Ralstonia solanacearum* causing Storage Rot of Ginger

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(Received: 26 July 2023; Revised: 28 August 2023; Accepted: 22 September 2023; Published: 15 October 2023)
(Published by Research Trend)

ABSTRACT: Ginger (*Zingiber officinale* Rosc.), a tropical plant in the Zingiberaceae family, is grown in nearly every country due to its culinary and therapeutic qualities. It is frequently used to season, taste, and add aroma to a variety of dishes during preparation, which raises the food's perceived value. A complex disease known as "storage rot" affects ginger which is caused by *Ralstonia solanacearum, Fusarium* spp., and *Pythium* spp. Storage rot causes up to 30% loss in value of ginger after harvest in Assam. In present investigation aqueous extracts of ten plants i.e. *Acorus calamus, Allamanda cathertica, Allium cepa, Allium sativum, Curcuma longa, Datura wrightii, Lasia spinosa, Laurus nobilis, Ocimum sanctum,* and *Piper betle* were tested against *R. solanacearum*. The growth of bacteria was significantly reduced by 20% in aqueous extracts of *A. sativum* and the rest of botanicals were not able to restrict the growth of plant pathogens *in vitro*. Further testing at four distinct concentrations (5, 10, 15, and 20%), *A. sativum* showed inhibition of 6.40, 9.40, 10.80, and 11.60 mm against *R. solanacearum*. Thus, *A. sativum* can be employed as a crucial component of integrated disease management.

Keywords: Ginger, Ralstonia, botanicals, *Allium sativum*.

INTRODUCTION

Ginger (*Gingiber officinale Rosc.*) is a significant herb with a wide range of therapeutic and traditional uses. Indian Ayurvedic systems consider this precious spice to be a gift from God that cures ailments, which is well recognized for its pungent scent and pungency. Fresh ginger is utilized in the preparation of meat and vegetable dishes in various countries, particularly in China and India. Bacterial wilt (*Ralstonia solanacearum*) is the most important disease of ginger which is mainly prevalent in Kerala and the country's northeast part, particularly in Sikkim and West Bengal (Sarma and Anandaraj 2000). Post harvest rot (also regarded as storage rot) is a complex disease which includes number of fungal and bacterial plant pathogens as evident from many research findings (Dake and Edison 1989; Mukherjee *et al*., 1995; Ram and Thakore 2010; Sharma and Dohroo 2017). The post harvest loss of ginger in Assam has been reported to be 30% due to storage rot (Rahman *et al*., 2009). *Fusarium oxysporum* f. sp. *zingiberi*, *Pythium aphanidermatum* and *Pseudomonas solanacearum* are the main causes of ginger storage rot in India (Dake and Edison 1989; Dohroo, 1989). Carbendazim, Ridomil, Topsin M and other similar products are commonly used to effectively manage ginger storage rot (Grech and Swart 1990; Sharma and Dohroo 1991). Several studies on the treatment of post-harvest diseases have explored the use of plant extracts and microbial antagonists as viable chemical substitutes for synthetic fungicides (Chandhary, 2003; Sarvamangla, 1993). Sukanya *et al*., (2009) reported that methanol extracts of *Chromolaena odorata* showed inhibition zones against *Escherichia coli*, *Staphylococcus aureus*, *X. vesicatoria* and *R. solanaccearum*, respectively followed by chloroform extracts of the same plant leaf. Moussa *et al.* (2010) examined the efficacy of four distinct extracts derived from twenty-five indigenous plant species spanning seventeen families against two phytopathogenic bacteria i.e. *Erwinia carotovora* and *R. solanacearum*. These pathogens cause brown rot and soft rot disease that affect numerous significant cultivated plants. They found that *Myrtus communis* had the greatest capacity to fight these infections.

MATERIALS AND METHODS

Isolation of *Ralstonia solanacearum*

The diseased ginger rhizome was sliced into small (5 mm) pieces and surface sterilized in 4% sodium hypochlorite for 30 seconds in order to isolate the bacteria. To get rid of any remaining sodium hypochlorite, these were subsequently cleaned three times in sterilized distilled water. After that, the bits were crushed in three to four milliliters of sterilized distilled water and they were left to diffuse for five to ten minutes at room temperature. A loop filled with crushed leaches was aseptically spread onto Petri plates containing Triphenyl Tetrazolium Chloride (TTC or TZC) agar medium, and the plates were then incubated for two days at 28±1°C. Serial dilution was used to get individual colonies of the bacteria. After 36–48 hours, single colony was picked and streaked on TTC media in agar plates. The selected discrete colonies were sub

cultured on TTC agar slants for further studies. The bacterium was also stored by suspending a loop full of the bacterial growth in sterilized distilled water and kept at 4ºC for short term storage of about a month. Discrete colonies were purified by sub culturing successively for 4 times on nutrient agar. The cultures were maintained by periodic sub culturing on TTC agar slants.

To test the pathogenicity of the pathogen, healthy rhizomes were washed in tap water and then sanitized by immersion in 4 sodium hypochlorite followed by rinsing with sterile water two to three times. Rhizomes were inoculated with pure culture of bacteria by adopting pin-prick method (Patil *et al*., 2017).

Evaluation of plant extracts against *Ralstonia solanacearum*

Ten different plant parts were collected from different localities of Jorhat district of Assam for the preparation of aqueous extracts. Various botanicals used against *R. solanacearum* are listed in the Table 1.

Preparation of aqueous plant extracts

Fresh plants and their part listed in Table 1 were washed thoroughly in sterile distilled water. 100 grams of plant parts were ground in a pestle mortar by adding an equal quantity of sterilized distilled water (1:1 W/V). After grinding, the extracts were filtered through muslin cloth and centrifuged at 10,000 rpm for 20 minutes at room temperature and the supernatant was taken as a standard plant extract solution (100%). The method of preparation of plant extracts reported by Shekhawat and Prasad (1971) was followed with some modifications. The supernatant obtained after centrifugation was further filtered using a bacterial membrane filter (RanDisc, PVDF 0.22µm) and evaluated at 20 per cent concentration (for preliminary screening) using 'agar well diffusion method' (Perez *et al*., 1990) (Fig. 1 and 2).

Preliminary screening of botanicals against *Ralstonia solanacearum*

The antibacterial activity of plant extracts was evaluated using agar well diffusion method (Perez et *al*., 1990) with certain modifications. The preserved bacteria streaked in TTC media plate by back fourth motion of the needle. Then, the culture was inoculated in 20 milliliters of nutrient broth and kept for multiplication in BOD at 30ºC for 72 hours.

Table 1: List of botanicals used.

Sr. No.	Scientific name	Common name	Family	Plant part used
1.	*Acorus calamus* L.	Bach	Araceae	Rhizome
2.	*Allamanda cathertica* L.	Malatilata	Apocynaceae	Leaves
3.	*Alium cepa*. L.	Onion	Amaralydaceae	Bulb
4.	*Allium sativum* L.	Garlic	Alliaceae	Bulb
5.	*Curcuma longa* L.	Turmeric	Zingiberaceae	Rhizome
6.	*Datura wrightii* Regel	Datura	Solanaceae	Leaves
7.	*Lasia spinosa* L.	Chengmora	Lamiaceae	Leaves
8.	*Laurus nobilis* L	Bay leaf/Tejpat	Lauraceae	Leaves
9.	*Ocimum sactum* L.	Tulsi	Lamiaceae	Leaves
10.	*Piper betle* L.	Betel vine	Piperaceae	Leaves

The multiplied bacterial suspension was seeded to the cool molten TTC medium (100 ml). The inoculated medium was poured into the sterilized Petri plates and allowed to solidify. Wells (5 mm diameter) were punched in the agar after solidification and filled with 50 µl of aqueous extracts of botanicals along with sterile water as a control. Then plates were incubated at 28±1ºC for 72 hours in BOD incubator. After 72 hours, formation of an inhibition zone which is a sign of plant extract's ability to stop a pathogen's growth, was observed. Each plate's inhibitory zone was measured in millimeters, and the collected data underwent statistical analysis. Further testing was conducted with most promising botanicals at four different concentrations (5, 10, 15 and 20 percent) against *R. solanacearum*. After observing the growth inhibition of *R. solanacearum* at different concentrations of botanicals, the maximum inhibition concentration was determined statistically.

RESULTS AND DISCUSSION

Rhizome inoculated with *R. solanacearum* showed complete rotting and internal tissues became pulpy with a bad odour. When such rhizomes were compressed, the bacterial cell mass protruded. The current study's findings are consistent with those of Dake and Edison (1989), who documented a correlation between *Pseudomonas solanacearum* and ginger storage rot in Kerala.

Results indicated that out of ten botanicals only *A. sativum* showed inhibition (11.60 mm) and the rest of botanicals did not show any effect on growth of *R. solanacearum*. Chemical check Copper Oxychloride (CoC) (0.3%) showed inhibition zone of 14.60 mm (Table 2 and Fig. 1.).

Based on the performance of ten different botanicals on inhibition of *R. solanacearum* growth, *A. sativum* was selected for further studies. Results presented in table 3 indicated that per cent inhibition increased linearly with concentration. Statistical analysis of the data revealed that *A. sativum* at 20 per cent showed maximum inhibition zone of 11.60 mm followed by 10.80 mm at 15 per cent, and 9.40 mm at 10 per cent concentration. The lowest inhibition zone (6.40mm) was recorded at 5 per cent concentration (Table 3 and Fig. 2). In the present investigation aqueous extracts of ten botanicals were evaluated for their anti-bacterial activity and only *A. sativum* was found to be effective against *R. solanacearum*.

Table 2: Efficacy of different botanicals (20%) on growth of *Ralstonia solanacearum*.

Treatments	Mean diameter of inhibition zone (mm)
T1: *Acorus calamus*	0.00 (0.70)*
T2: *Allamanda cathertica*	0.00 (0.70)
T3: *Allium cepa*	0.00 (0.70)
T4: *Allium sativum*	11.60 (3.44)
T5: *Curcuma longa*	0.00 (0.70)
T6: *Datura wrightii*	0.00 (0.70)
T7: *Lasia spinosa*	0.00(0.70)
T8: *Laurus nobilis*	0.00 (0.70)
T9: *Ocimum sanctum*	0.00 (0.70)
T10: *Piper betel*	0.00 (0.70)
T11: CoC (0.3%)	14.60 (3.86)
T12 : Control	0.00 (0.70)
SEd (±)	0.02
CD (p = 0.05)	0.03

* Data in parenthesis represents square root transformation

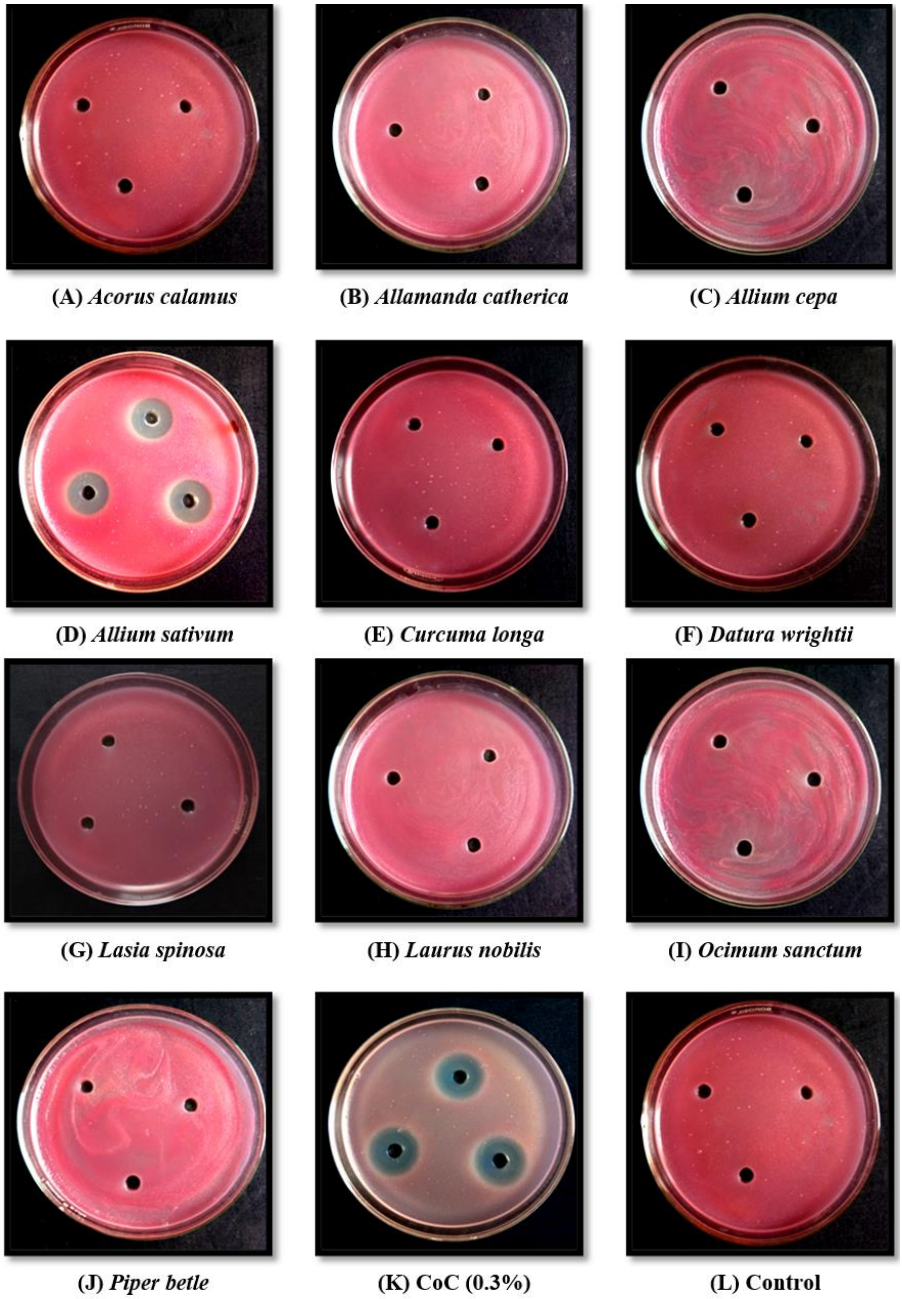

Fig. 1. Efficacy of different botanicals (20%) on growth of *R. solanacearum* (after 72 hrs of inoculation).

Table 3. Efficacy of *Allium sativum* (5, 10, 15 and 20%) on growth of *Ralstonia solanacearum.*

Concentration (%)	Mean diameter of inhibition zone (mm)
5	6.40 (2.62)*
10	9.40 (3.14)
15	10.80 (3.36)
20	11.60 (3.47)
CoC (0.3%)	14.60 (3.88)
Control	00 (0.70)
SEd (±)	0.06
CD (p=0.05)	0.11

* Data in parenthesis represents square root transformation

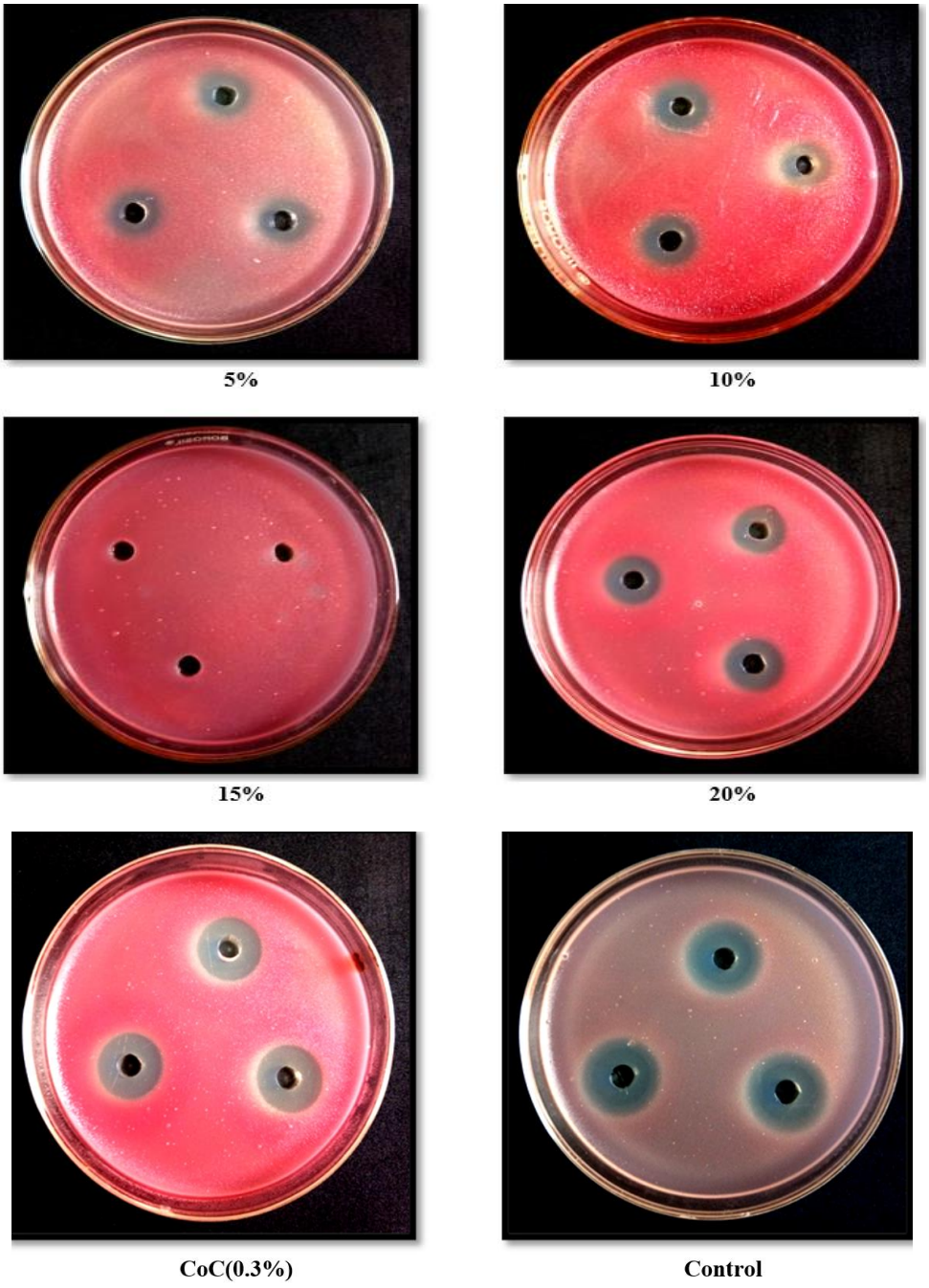

Fig. 2. Efficacy of *Allium sativum* (5, 10, 15 and 20%) on growth of *R. solanacearum* (after 72hrs of inoculation).

The results of the *in vitro* test of the present study are in agreement with those reported by earlier workers (Khan *et al*., 1974; Hannudin and Djantnika 1989) who tested garlic (*Allium sativum*) and onion (*Allium cepa*) extracts against *R. solanacearum* and found that only garlic had an inhibitory effect on the growth of *R. solanacearum* under *in vitro* conditions, but the onion extract had no effect. *A. sativum* having inhibitory effect due to steroid, saponin, alkaloids, flavonoids, glycosides, phenolic compounds and terpenoids present in the cloves of *A. sativum* (Divya *et al.,* 2017), and plants that are rich in above phytochemicals possess antimicrobial activity against a number of micro organisms (Adebajo *et al*., 1983; Gandhiraja *et al*., 2009). Similarly, Kumar *et al*. (2019ab) reported a strong inhibitory effect of *A. sativum* against fungal pathogens i.e. *Fusarium oxysporum* and *Pythium aphanidermatum* associated with the storage rot of ginger.

On the other hand, the non inhibitory effect of botanicals against *R. solanacearum* might be due to the use of water extract for the study. Water extracts did not inhibit the growth of any of the tested bacteria (*Klebsiella pneumoniae* 2, *Escherichia coli* 3, and *Staphylococcus aureus* 3) However, ethanol and methanol extracts were found to be more potent, capable of exerting significant inhibitory activities against the majority of the tested bacteria (Dahiya and Purkayastha 2012). Joshi *et al.* (2009) discovered that ethanol extracts demonstrated superior efficacy over water extracts against bacterial plant pathogens.

CONCLUSION

In the present study, out of ten botanicals tested, only *A. sativum* exhibited inhibition of *R. solanacearum*. The most effective botanicals were further tested against pathogenic bacteria at 5, 10, 15 and 20 per cent concentration respectively. *A. sativum* at 20 per cent showed the maximum inhibition zone followed by 15 and 10 per cent concentration. The lowest inhibition zone recorded at 5 per cent concentration. Among the three concentrations, tested for aqueous extract of each botanical, 20 per cent was found to be most effective over 15, 10 and 5 per concentrations. Thus, *A. sativum* has potential as a substitute for just using synthetic fungicides and can be employed as a crucial component of integrated disease management.

FUTURE SCOPE

Exploration of botanicals with higher efficacy than those considered under the study. There is a need for the identification and characterization of antimicrobial components present in effective botanicals which will be useful in developing plant based commercial formulation in the near future. Studies are needed to elucidate information on the exact role of the individual chemical constituent of the botanicals on suppression of the pathogen.

Acknowledgement. The author expresses gratitude to Dr. Ashok Bhattacharyya, his major advisor, for his unwavering support throughout the research project. Additionally, the author acknowledges Head of Plant Pathology Department Assam Agricultural University's for providing all essential laboratory facilities.

Conflict of Interest. None.

REFERENCES

Adebajo, A. O.; Adewumi, C. O. and Essien, E. E. (1983). Antiinfective agent of higher plants. International Symposium of Medium Plants. 5th ed. University of Ife, Nigeria. pp. 152-158.

Choudhary, R. F. and Patel, R. L. and Chaudhari, S. M. and Pandey, S. K. and Singh, B. (2003). *In vitro* evaluation of different plant extracts against *Alternaria alternata* causing early blight of potato. *J. Indian Potato Assoc. 30*, 141-142.

Dahiya, P. and Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian J. Pharm. Sci*., *74*, 443.

Divya, B. J., Suman, B., Venkataswamy, M. and Thyagaraju, K. (2017). A study on phytochemicals, functional groups and mineral composition of *Allium sativum* (garlic) cloves. *International Journal of Current Pharmaceutical Research*, *9*, 42-45.

Dake G. N. and Edison, S. (1989). Association of pathogens with rhizome rot of ginger in Kerala. *Indian Phytopathol*., *42*, 116-119.

Dohroo, N. P. (1989). Seed transmission of pre-emergence rot and yellows in ginger. *Pl. Dis. Res*., *4*, 73-74.

Gandhiraja, N., Sriram, S., Meenaa, V., Srilakshini, J. K., Sasikumar, C. and Rajeswari, R. (2009). Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L. against selected microbes. *Ethnobot. Leaflets*, *13*, 618-624.

Grech, N. M. and Swarts, D. H. (1990). Post-harvest application of fungicides for control of fungal decay of ginger rhizomes stored under simulated low temperature shipping conditions. *Phytophylactica, 22*, 457-458.

Hannudin and Djantnika, I. (1989). The effect of some plant extracts on bacterial wilt (*P. solanacearum*) growth *in vitro*. *Bull. Penelitian Hortikultura*, *14*, 12-14.

Janisiewiez, W. J. and Korsten, L. (2002). Biocontrol of post harvest disease of fruits. *Annu. Rev. Phytopathology*, *40*, 411-441.

Joshi, B. and Lekhak, S. and Sharma, A. (2009). Antibacterial property of different medicinal plants: *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum armatum* and *Origanum majorana*. *Kathmandu Univ. J. Sci. Eng. Tech*., *5*, 143-150.

Khan, A. N. A. (1974). Studies on *Pseudomonas solanacearum* (E. F. Smith) causing wilt of brinjal, potato and tomato in Mysore state. *Mysore J. Agric. Sci.*, *8*, 478-479.

Kumar, S., Bhattacharyya, A., and Kashyap, P. (2019a). Morphogenetic characterization of *Fusarium oxysporum* associated with storage rot of ginger in Assam and in vitro evaluation of botanicals. *Journal of Pharmacognosy and Phytochemistry*, *8*(6), 2513-2518.

Kumar, S., Bhattacharyya, A., Savani, A. K., and Gogoi, S. (2019b). Antifungal Activity of Some Local Botanicals of Assam against *Pythium aphanidermatum* Inciting Storage Rot of Ginger. *Int. J. Curr. Microbiol. App. Sci*., *8*(11), 528-535.

Moussa, A. M. and Emam, A. M. and Mohamed, M. A. and Diab, Y. M. (2010). *In vitro* evaluation of some Egyptian plants against the rot bacteria and spider mite and isolation the active constituent(s) from *Myrtus communis* leaves. *Int. Food Res. J., 17*, 287-294.

Mukherjee, P. K. and Thomas, P. and Raghu, K. (1995). Shelf life enhancement of fresh ginger rhizomes at ambient temperature by combination of gamma radiation, biocontrol and closed polyethylene bag storage. *Appl. Biol., 2,* 375-384.

Patil, A. G.; Ambadkar, C. V.; Kashid, V. S. and Navgire, K. D. (2017). Standardization of methods for pathogenicity of pomegranate bacterial blight caused by *Xanthomonas axonopodis* pv. *punicae*. *J. Pharmacogn. Phytochem., 6*, 1763-1765.

Perez, C. (1990). Antibiotic assay by agar-well diffusion method. *Acta. Biol. Med. Exp*., *15*, 113-115.

Rahman, H.and Karuppaiyan, R. and Kishore, K. and Denzongpa, R. (2009). Traditional practices of ginger cultivation in Northeast India. *Indian J. Tradit. Knowl., 8,* 23-28.

Ram, L. and Thakur, T. L. (2010). Association of different fungi under traditional storage of ginger rhizomes. *J. Plant Pathol*., *3*, 678-679.

Sarma, Y. R. and Anandaraj, M. (2000). Disease of spices crops and their management. Ind. J. *Arecanut Spices Med. Pl., 2*, 8-20.

Sarvamangla, H. S. (1993). Evaluation of plant extract for the control of fungal disease of blackberry. *Indian Phytopathol., 46*, 398-401.

Sharma, S. K., and Dohroo, N. P. (1991). Post harvest management of Rhizome rot *(Fusaruim oxysporum* f. sp. *zingiberi Trujillo)* of ginger through chemical and antagonist. *Indian Cocoa Arecanut Spices J., 14*, 150-152.

Sharma, S., Dohroo, N. P., Veerubommu, S., Phurailatpam, S., Thakur, N. and Yadav, A. N. (2017). Integrated Disease Management of Storage Rot of Ginger (*Zingiber officinale*) caused by *Fusarium* sp. in Himachal Pradesh, India. *Int. J. Curr. Microbiol. App. Sci*., *6*, 3580-3592.

Shekhawat, P. S. and Prasad, R. (1971). Antifungal properties of some plant extracts and their inhibition of spore germination. Indian *Phytopathol., 24*, 800–802.

Sukanya, S. L., Sudisha, J., Hariprasad, P., Niranjana, S. R., Prakash, H. S., Fathima, S. K. (2009). Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. *Afr. J. Biotechnol., 8*, 106-112.

How to cite this article: Sonal Kumar, Ashok Bhattacharyya, Pranab Dutta and Jayant Sahu (2023). Evaluation of plant extracts against *Ralstonia solanacearum* causing storage rot of Ginger. *Biological Forum – An International Journal, 15*(10): 365-370.