International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
www.chemijournal.com
IJCS 2021; 9(1): 3459-3461

Received: 03-10-2020
Accepted: 09-12-2020

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In vitro evaluation of botanicals against *Pestalotiopsis clavispora* causing crown rot of strawberry

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DOI: https://doi.org/10.22271/chemi.2021.v9.i1aw.11768

Abstract
Strawberry (*Fragaria Xananassa* Duch.) is an important fruit crop grown in the Dangs district of Gujarat. Crown rot emerged as one of the major disease of strawberry. A total of seven commonly available botanical extracts were evaluated against the growth of *Pestalotiopsis clavispora* under *in-vitro* condition. Results of the *in vitro* evaluation of various botanicals against *Pestalotiopsis clavispora* revealed that all the test botanicals significantly inhibited mycelial growth of *Pestalotiopsis clavispora*, over untreated control. Garlic was found most effective followed by neem, ginger, turmeric, Arjun, Ardusiand tulsi.

Keywords: Botanicals, crown rot, *in vitro* and *Pestalotiopsis clavispora*

Introduction
Strawberry (*Fragaria Xananassa* Duch.) is one of the most important soft fruit of world and occupies an important place amongst small fruits. It occupies an area of 395,844ha with a total production of 9,223,815MT (FAO, 2017). In India, strawberry cultivation is confined only to hilly tracts of Himachal Pradesh, Uttarakhand, Jammu Kashmir, West Bengal, Haryana, Uttar Pradesh, Punjab, Maharashtra and Karnataka have their own identity for strawberry production occupied an area of 1000ha with a total production of 5000MT (Anon., 2018a) [1]. In Gujarat state, now a days Dang district alone is the main area of strawberry cultivation under the many villages having over an area of 23ha with production of strawberry per week is pegged to 1,750kg (Anon., 2018b) [2]. The area under its cultivation has increased considerably in the last 1 to 2 years with the awareness among fruit growers and better transport and storage facilities. Diseases are the major constraints in economic production of strawberry. Many pathogen of strawberry have been recorded over the world (Dung *et al.* 2016) [4]. However, crown rot is caused by several pathogens *viz*., *Pestalotiopsis* sp. *Phytophthora cactorum*, *Colletotrichum acutatum*, *C. gloeosporioides* and *C. fragaria*. Among them, *Pestalotiopsis* spp. causes crown rot that poses a serious threat to the crop inflicting qualitative and quantitative reduction in strawberry production (Dung *et al.*, 2016) [4].

Materials and Methods
Isolation of pathogens from infected plants
Fresh infected leaves and crown of strawberry were used to isolate the pathogen. The infected areas were cut into small pieces in such a way that each piece consist of healthy as well as infected portion. The pieces were surface sterilized with 0.1 per cent (1g/l) mercuric chloride ($HgCl_2$) solution for 60seconds followed by three subsequent washings with distilled sterile water and then aseptically transferred to sterilized disposable plastic 90mm diameter Petri plates containing 20ml Potato dextrose agar (PDA) medium and these Petri plates incubated at average mean room temperature (25±2 ºC). The fungal hyphae developed from infected tissues after 48hours were sub cultured aseptically on PDA slants or Petri dishes containing PDA. The pure cultures thus obtain by hyphal tip method that was further maintained by frequent sub-culturing and the isolate was used for further studies.

In vitro Evaluation of Botanical extracts

Botanical extracts of seven plant species belonging to different families were evaluated against *P. clavispora* by Poisoned Food Technique as suggested by (Grover and Moore, 1962) [5].

Table 1: List of different botanicals tested for their efficacy against the *P. clavispora in vitro* (10% concentration)

Treatment No.	Common name	Botanical name	Part used
T_1	Garlic	*Allium sativum* L.	Cloves
T_2	Tulsi	*Ocimum sanc*tum L.	Leaves
T_3	Ardusi	*Adhatodavasika* Nees.	Leaves
T_4	Neem	*Azadirachta indica* L.	Leaves
T_5	Ginger	*Zingiber officinalis* Rosa	Rhizome
T_6	Turmeric	*Curcuma longa* L.	Rhizome
T_7	Arjun	*Terminalia arjuna*	Bark
T_8	Control	-	-

Healthy fresh plant parts i.e., leaves, cloves, rhizomes and bark were taken (Table 1) and washed thoroughly with clean water and finally rinsed with sterilized distilled water. Fifty grams (50g) of respective plant parts (Table 1) were minced with the help of grinder by adding 50ml sterilized water. The extracts were filtered through double layers sterilized muslin cloth and collected in 150ml conical flasks and plugged with non-absorbent cotton. The filtered phytoextracts were autoclaved at 1.2kg/cm^2 pressure for 20minutes. The sterilized extracts were individually added in previously sterilized PDA at 10 per cent (i.e. 2ml phytoextract + 18ml PDA per plate) in the conical flasks and mixed thoroughly at the time of pouring in the previously sterilized Petri plates. All the plates containing phytoextracts were inoculated by placing the 5mm mycelial disc from efficiently grown seven days old pure culture of Pestalotiopsis spp. with sterilized forceps and incubated at average mean temperature (25±2ºC). Three repetition of each treatment were maintained and the plates without phytoextract served as control. The observations on colony diameter (mm) were recorded periodically until any of the plate fully covered with the growth of Pestalotiopsis spp. and the per cent growth inhibition (PGI) was calculated as per formula given by Vincent (1947).

$$PGI = \frac{100\,(DC-DT)}{DC}$$

Where

PGI = Per cent growth inhibition

DC = Average diameter of mycelial colony in control set (mm)

DT = Average diameter of mycelial colony of treated set (mm)

Results and Discussion.

The results presented in Table 2, Plate 1 and depicted graphically in Fig. 1 revealed that all the phytoextracts tested were significantly superior over the control in checking the growth of the pathogen. Among the effective phytoextracts, least mycelial growth of *Pestalotiopsis clavispora* was recorded in the extract of garlic (42.00mm) which was proved to be significantly superior. Next best in order of merit was neem (46.67mm) followed by ginger (51.00mm) which was statistically at par with turmeric (51.33mm) and rest of phytoextracts showed comparatively less inhibitory effect.

The extract of garlic produced maximum growth inhibition (47.50%) followed by neem (41.66%), ginger (36.25%), turmeric (35.84%), arjun (20.41%), ardusi (9.59%) and tulsi (3.34%).

Table 2: Inhibitory effect of different botanicals against *P. clavispora in vitro*

Sr. No.	Common name	Botanical name	Average colony diameter(mm)	Growth inhibition over control (%)
T_1	Garlic	*Allium sativum* L.	42.00	47.50
T_2	Tulsi	*Ocimum sanc*tum L.	77.33	03.34
T_3	Ardusi	*Adhatodavasika* Nees	72.33	09.59
T_4	Neem	*Azadirachta indica* L.	46.67	41.66
T_5	Ginger	*Zingiber officinalis* Rosa	51.00	36.25
T_6	Turmeric	*Curcuma longa* L.	51.33	35.84
T_7	Arjun	*Terminalia arjuna*	63.67	20.41
T_8	Control	-	80.00	-
S. Em. ±			0.95	
CD at 5%			2.84	
CV %			2.72	

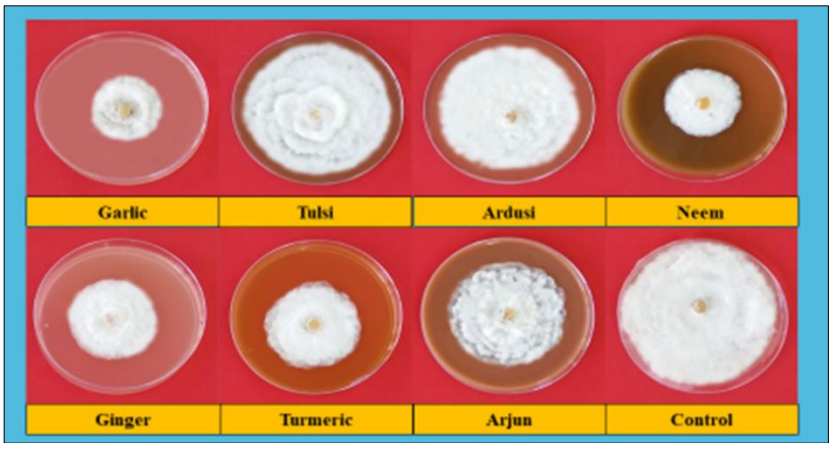

Plate 1: Evaluation of different botanicals against *P. clavispora in vitro*

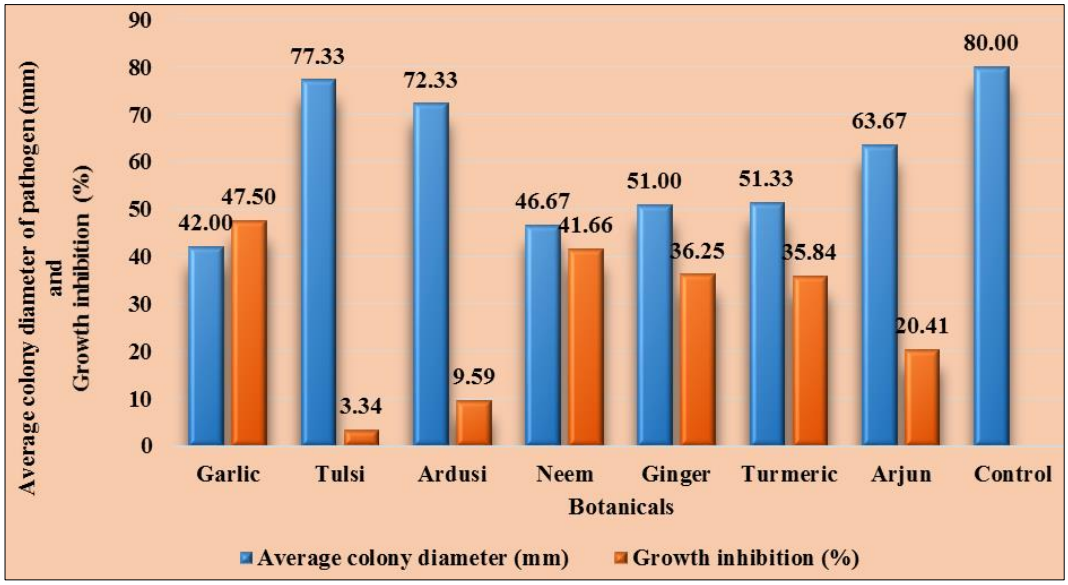

Fig 2: Inhibitory effect of different botanicals against *P. clavispora in vitro*

From this experiment, it is clear that extracts of garlic (*Allium sativum* L.), neem (*Azadirachta indica* L.), ginger (*Zingiber officinalis* Rosa) and turmeric (*Curcuma longa* L.) may have some strong toxic principle present in their extract which directly affects the growth of *Pestalotiopsis clavispora*, the causal agent of crown rot of strawberry.

In our present study the garlic, neem, ginger and turmeric were found most effective for controlling *P. clavispora* under *in vitro* condition. The findings were nearly similar with reports made by Rokade (2009) [7] and Patil (2012) [6], whereas Devi *et al.* (2017) [3] also showed the garlic clove extracts as the most effective in mycelial growth inhibition of *Pestalotiopsis mangiferae.*

Conclusion

Results concluded that the extract of Garlic (*A. sativu*m L.) was proved excellent in inhibiting mycelial growth of *Pestalotiopsis clavispora*. The next best phyto extracts were neem (*Azadirachta indica* L.), ginger (*Zingiber officinalis* Rosa) and turmeric (*Curcuma longa* L.).

Acknowledgement

The authors are thankful to Head, Department of Plant Pathology and Directorate of Research & Dean PG Studies, NAU, Navsari, (Gujarat) for providing all the facilities to conduct work.

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