International Journal of Modern Agriculture, Volume 9, No.4, 2020
ISSN: 2305-7246

Shelf-Life Improvement In Pineapples By Using Plant Extracts To Control Ceratocystis Paradoxa.

Dr. Khumukcham Susheela Devi[1] and Dr. M. Damayanti Devi[2]

[1]Department of Botany, DM College of Science, Dhanamanjuri University, Imphal

[2]Department of Life Sciences, Manipur University, Imphal

Keywords: pineapple, *Ceratocystis paradoxa, Xanthium strumarium* extract, antifungal activity

Abstract:

Ceratocystis paradoxa, a fungus causes soft-rot of pineapples. The high temperature and humid condition prevailing during the peak fruiting season (June-July) promote infection by pathogenic microorganisms. In the recent past, angiospermic plants have proved to be useful sources of fungitoxic substances that are rather harmless compared to synthetic chemical fungicides which often impose undesirable side effects. The present investigation also reveals that plant extracts can be used in controlling *C. paradoxa. Xanthium strumarium* was the most effective in inhibiting mycelial growth *in vitro*. The effectiveness of various extracts against *C. paradoxa* was in the decreasing order of *Phlogacanthus thyrsiflorus, Toona ciliata, Vitex negundo, Azadirachta indica, Eupatorium birmanicum, Ocimum sanctum* and *Leucas aspera.* Extracts of *Gynura cusimba* and *Ocimum canum* showed poor fungitoxicity. Millipore filter-sterilized extracts had a more inhibitory effect on the fungus than the autoclaved sample.If pineapple juice was added to the medium, the growth of *C. paradoxa* increased with increasing concentration of the juice. Xanthium extract also inhibited the growth of the fungus in this case. Treatment of pineapple fruits with *X. strumarium* extract reduced the severity of the disease. Since the extract has significant preventive activity against soft-rot disease in pineapples, there is possibility of using such an extract as a fungitoxic agent in post-havest fruit technology development.

Introduction:

Pineapple [*Ananas comosus* (L.) Merr. cv. Queen] is a widely cultivated cash crop of Manipur, the north-easternmost state of India, which is bounded on the north by Nagaland, on the south by Myanmar and Mizoram, on the east by Myanmar and on the west by Assam. Manipur lies between 23º80'N and 25º68ºN latitude and 93º03'E and 94º80'E longitude. The total area of the state is 22,327 sq.km.

A huge quantity of pineapple in Manipur is wasted predominantly due to infection by an ascomycete fungus, *Ceratocystis paradoxa* (Dade)-Moreu. Other fungal pathogens like *Penicillium purpurogenum* Stoll. and *Aspergillus niger* V. Tiegh also damage the fruits (Py *et al*., 1987; Damayanti *et al*., 1992). The high temperature and humid condition prevailing during the peak fruiting season (June-July) promote the pathogenic microorganisms.

In the recent past, the angiospermic plants have been proved to be useful sources of fungitoxic substances that are rather harmless compared to synthetic chemical fungicides, which often impose undesirable side effects. The use of plant extracts to reduce the incidence of various plant diseases has been reported (Egawa *et al*., 1977; Mangamma and Sreeramulu, 1991; Sarvamangla *et al*., 1993). The identification of plants whose extracts can be used for controlling *C. paradoxa* will be a cheap and effective alternative to fungicides which may pose health hazard to us.

The present study investigates the plant extracts having antifungal activity against *C. paradoxa* in order to check post-harvest loss. The technique employed in this study is the poisoned food technique.

Material and methods

Fig 1. *Xanthium strumarium*

Leaf extracts of the following plants were prepared after washing them thoroughly and crushing them – *Xanthium strumarium* (Fig. 1), *Ocimum sanctum, Gynura cusimba, Leucas aspera, Azadirachta indica, Toonia ciliata, Ocimum canum Eupatorium birmanicum, Vitex negundo* and *Phlogacanthus thyrsiflorus*.

The leaf extracts were filtered and then centriguged at 3,500 rpm for 20 minutes. The supernatant for each extract was collected separately. 5ml of supernatant of each leaf extract was taken and 25 ml of potato dextrose agar (PDA) was added. After this steam sterilization was carried out and then plated using sterilized Petri plates of 15 cm diameter. A control was maintained by taking 30ml of potato dextrose agar medium without any supernatant or extract. The plant extract which was found to be most promising was used for further tests.

Fresh leaves of *X. strumarium* were divided into three lots weighing 1g each. Two lots were oven-dried separately. One of the dried samples was grounded into powder while the other portion was burnt into ash. The fresh leaves (1g) were crushed. The oven-dried powder/ash/crushed fresh leaves were extracted separately by steaming in 100ml of distilled water for 30 min. and filtered and centrifuged as before. Different volumes of the superanatants from these extracts were made upto 30 ml with PDA. After steam sterilization, they were plated in sterilized Petri plates. In the same way, the oven-dried powder/ash (1g) was also extracted and processed.

Single spore culture of *C. paradoxa* (isolated from diseased pineapple fruits) (Fig. 2 and 3) grown and maintained on PDA was used for the experiments A 0.5. cm diameter agar disk, taken from 48-h-old sporulated *C. paradoxa* culture was placed with the fungal side downward in the centre of each plate which was incubated in the dark at 28-38ºC. Radial growth was determined by measuring the colony size along two diameters at right angles at various time intervals. Three replicates for each treatment were prepared. The experiments were repeated three times. Plant extracts showing more than 70% inhibition in mycelial growth compared with the control were taken as effective extracts.

Fig. 2 Pineapple fruits with *C. paradoxa*

In order to study the effect of *X. strumarium* in reducing post-harvest fruit loss due to *C. paradoxa*, 100 pineapple fruits of uniform weight (600-650g) and size were selected. These were then divided into two groups of which the first was kept as control. The second group of pineapples was treated with leaf extract of *X. strumarium* to see the effectiveness in controlling *C. paradoxa*. After treatment, both the groups were stored at 25-29ºC and 90-97% relative humidity. The experiments was replicated three times.

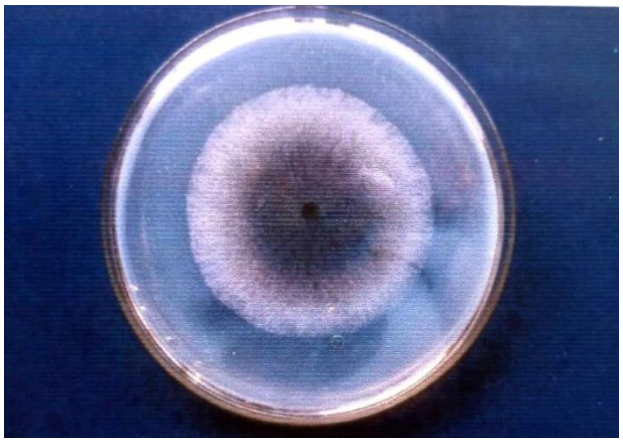

Fig. 3. Colony of *C. paradoxa*

Results:

Of the ten plant extracts tested, *X. strumarium* was found to be the most effective in reducing mycelial growth. The leaf extract showed significant effectiveness in controlling the fungus. Leaf extracts of *O. sanctum, L. aspera, A. indica, T. ciliata, E. birmanicum, V. negundo* and *P. thyrsiflorus* were found to be less effective in controlling mycelical growth. Leaf extracts of *G. cusimba* and *O. canum* did not exhibit 70% inhibition and hence considered ineffective. (Table 1 and 2).

Table 1 Effective of plant extracts on radical growth of *C. paradoxa* after 36 h incubation.

Source of extracts	**Radial growth (mean ± SE)**	**Effectiveness (70% inhibition)**
Control (PDA)	6.30 ± 0.05 (0.20)	-
Gynura cusimba	6.76 ± 0.05 (0.23)	-
Ocimum canum	4.43 ± 0.05 (0.15)	-
Leucas aspera	4.43 ± 0.05 (0.15)	+
Ocimum sanctum	4.30 ± 0.06 (0.11)	+
Eupatorium birmanicum	3.60 ± 0.05 (0.10)	+
Azadirachta indica	2.90 ± 0.04 (0.12)	+
Vitex negundo	2.60 ± 0.05 (0.11)	+
Toona ciliata	2.16 ± 0.06 (0.10)	+
Phlogacanthus thyrsiflorus	2.10 ± 0.05 (0.05)	+
Xanthium strumarium	0.5 (-)	+

Growth rate in cm/h is shown in parentheses. Effectiveness of inhibition: -, <70%; +. >70%

Table 2 Effect of various concentrations of extracts of *X. strumarium* on radial growth of C. paradoxa after 36 h incubation.

Treatment (ml)	**Radial growth *X. strumarium***	**(mean ± SE):**
0.1	4.70 ± 0.02	(0.17)
0.5	2.20 ± 0.04	(0.06)
1.0	1.10 ± 0.04	(0.06)
2.0	0.50	(-)
3.0	0.50	(-)
4.0	0.50	(-)
5.0	0.50	(-)

The final volume for each treatment was adjusted to 30 ml with PDA. Growth rate in cm/h is shown in parentheses.

Table 3 Effect of Millipore filter-sterilized extracts (1g/100 ml) of *X. strumarium* using distilled water as extractants on radial growth of *C. paradoxa* after 36 h incubation.

Treatment (5 ml each)	**Radial growth**	**(mean ± SE):**
PDA	6.30 ± 0.03	(0.20)
Distilled water-extract	3.50 ± 0.05	(0.20)

The final volume for each treatment was adjusted to 30 ml with PDA. Growth rate in cm/h is shown in parentheses.

The Milipore filter-sterilized extract was more effective in controlling fungal growth than autoclaved samples (Table 3) when different concentrations of pineapple juice was added to the medium, radial growth of the fungus increased with increasing concentration of juice. When leaf extract of *X. strumarium* was also added, fungal growth decreased proportionally (Table 4).

Table 4 Effect of leaf extract (without any extractant) of *X. strumarium* on radial growth of *C. paradoxa* in the presence of pineapple juice after 36 h incubation.

Treatment (ml): PDA with	Pineapple juice and	Extract	Radial growth (mean ± SE)	
30	0	0	6.30 ± 0.03	(0.20)
29	1	0	7.00 ± 0.06	(0.24)
27	3	0	7.40 ± 0.04	(0.25)
25	5	0	7.80 ± 0.05	(0.26)
24	5	1	4.72 ± 0.03	(0.18)
23	5	2	3.75 ± 0.05	(0.10)
22	5	3	2.60 ± 0.02	(0.10)
21	5	4	1.20 ± 0.04	(0.05)
20	5	5	0.50	(-)

Table 5 and Fig. 3 Effect of leaf extract of *X. strumarium* on disease incidence (%) in pineapples caused by *C. pardoxa*. Each value is an average of three replicates.

Table 5

Sample	Storage duration (Days)									
	10	12	14	16	18	20	22	24	26	28
Extract (-)	9.3	21.3	36.7	46.7	65.3	100				
Extract (+)	-	8.2	16.1	21.5	27	34	42.7	50	56.5	60.7

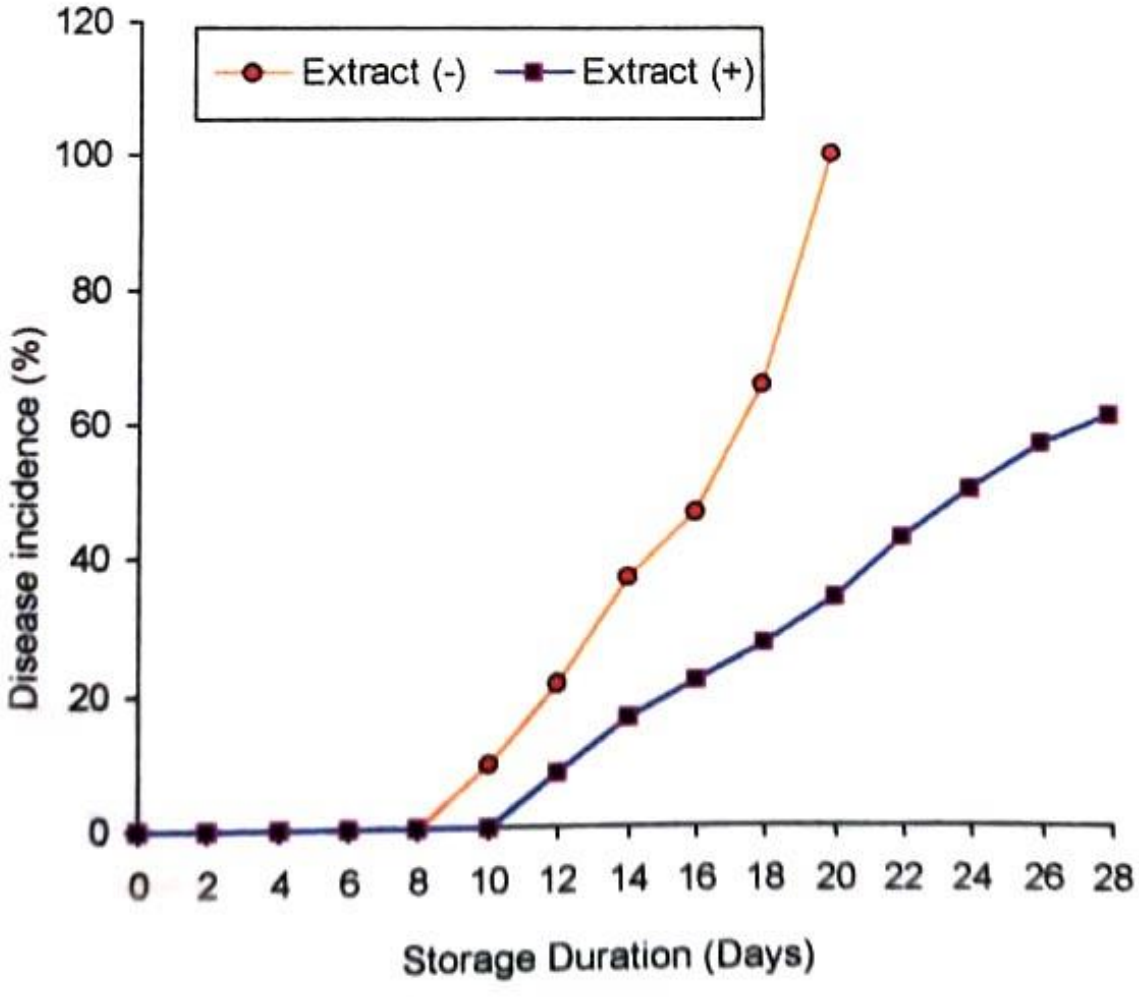

Fig. 6

Discussion:

The present investigation reveals that plant extracts can be used in controlling *C. paradoxa*. Leaf extracts of *Xanthium strumarium* was found to be effective in reducing fruit loss due to *C. paradoxa*. *In vitro* experiment also showed that mycelial growth of the fungus was significantly reduced when leaf extract of *X. strumarium* was used. Jawad *et al* (1988) have identified xanthanol as an anti-

microbial agent. However, further confirmation as the agent effect against *C. paradoxa* is required. Use of plant extracts for controlling fungal diseases provide an inexpensive approach for reducing post harvest fruit loss.

References:

1. DAMAYANTI M., Sharma G.J. and Kundu S.C. 1992. Gamma radiation influences post – harvest disease incidence of pineapple fruits. *Hortscience* 27 807-8.

2. EGAWA H., Tsutsui O., Tatsuyama K. And Hatta T. 1977. Antifungal substances found in leaves of *Eucalyptus spp. Experientia* 33 889-90.

3. JAWAD A., Mahmoud M.J. and Al-Naib A. 1988. Antifungal activity of *Xanthium strumarium* extracts. Fitoterapia 59 220-1.

4. MANGAMMA P. and Sreeramulu A. 1991. Garlic extract inhibitory to growth of *Xanthomonas campestris* pv. *vesicatoria. Ind. Phytopath*. 44 372-4.

5. Py. C., Lacoeuilhe, J.J. and Teisson, C, 1987. *The pineapple: Cultivation and uses. Techniques Agricoles et Productions Tropicales.* G.P. Maison neuve et Larose, Paris. pp 205-209.

6. SARVAMANGLA H.S., Govindaiah and Datta R.K. 1993. Evaluation of plant extracts for the control of fungal diseases of mulberry. *Ind. Phytopath.*46 398-401.