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Efficacy of agrochemicals and botanicals against phyto-pathogenic fungi causing damping-off disease in brinjal

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Abstract
One of the major problems for production of brinjal crop is damping-off disease and it is caused by the pathogens like *Rhizoctonia solani*, *Phytophthora* sp., and *Fusarium* sp. The present studies were undertaken on isolation, characterization, pathogenicity test, *in-vitro* bioassay of fungicides, and botanicals. The *Rhizoctonia solani* and *Fusarium pallidoroseum* were isolated and identified from the infected brinjal seedlings. In *in-vitro* condition Carboxin + Thiram (0.2%), Tebuconazole (0.1%), and Hexaconazole (0.1%) recorded inhibition of *Rhizoctonia solani* and *Fusarium pallidoroseum* growth by 100%. The Chlorothalonil (0.2%) was the least effective fungicide against *R. solani* and *F. pallidoroseum*. Among the plant extracts *Azadirachta indica* and *Allium sativum* were significantly effective in reducing the growth of *R. solani*, and *F. pallidoroseum* as compared to control.

Keywords: Botanicals, brinjal, damping-off, fungicides

Introduction
Damping-off is one of the destructive diseases in the greenhouse, field and nursery, affecting germinating seeds and young seedlings in solanaceous crops. However, the damping-off is mainly caused by *Fusarium* sp., *Rhizoctonia* sp., *Pythium* sp. and *Phytophthora* sp. since these pathogens are the most frequently associated with damping-off and are considered the most important causal agents.
Brinjal is an important and widely consumed vegetable crop of India grown round the year. It is stated that plant diseases pose a threat to crop production, because, at least, 10% of global food production is lost to plant diseases (Strange and Scott, 2005) [5]. Brinjal is attacked by fungal, bacterial, viral and insect pests and cause significant economic loss by reducing yield and marketable value of the crop. More than ten diseases are reported on brinjal in this country, of which only a few are important. Damping off caused by *Fusarium* sp., *and Rhizoctonia* sp. Are very severe in nursery (Horst, 2013) [2].

Materials and Methods
During the present studies on damping-off of brinjal experiments were undertaken at Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar during 2019-20.
The survey was carried out in the experimental plots of All India Coordinated Research Project on Vegetables, Odisha University of Agriculture & Technology, Bhubaneswar and Central Horticultural Experimental Station, Bhubaneswar. For the survey random patches in nursery plot on brinjal. After proper observations in each lot total number of plants were calculated and among them number of seedlings showing damping off symptom were counted on the basis of visual symptoms. In each verity ten observations were taken. From the survey the percent of disease incidence was calculated. The average of all the plots was taken to find out the final result.

$$\text{Percent disease incidence (PDI)} = \frac{\text{Number of infected plants}}{\text{Total number of plants assessed}} \text{X100}$$

The brinjal seedlings showing typical symptoms of damping off were collected from the fields, washed thoroughly with distilled water, blot dried and cut with sharp sterilized blade into small bits (5 mm) these bits were then surface sterilized with 0.1 per cent aqueous solution of mercury chloride ($HgCl_2$) for two minutes, washed by giving three successive changes with sterile distilled water in glass petriplates to remove traces of mercuric chloride and blot dried.

These surface sterilized bits were inoculated aseptically on autoclaved and cooled PDA medium in sterilized glass petriplates under aseptic conditions of laminar air flow cabinet and inoculated in BOD incubator at 27±2 °C temperature. Within 3-4 days of incubation pale white mycelia mat was developed. Applying hypha tip and single spore isolation technique, the test pathogen was transferred aseptically on the PDA slants (Ainsworth, 1961) [8]. Through frequent sub-culturing, the test pathogen was purified and the pure culture were maintained on PDA slant in test tubes and maintained in refrigerator for further studies. The cultures were identified at Department of Plant Pathology, Indian Agricultural Research Institute (IARI) and New Delhi. Koch's postulates were demonstrated for all isolated pathogen.

The healthy seeds of twenty numbers were shown in poly bags (6"x10") containing sterilized soil (1kg each) with sterilized soil and mixed with well decomposed FYM and fertilizers. The healthy seedlings of 20 days old were selected for the test. Watering was done thrice in a week. The base portions of the plants were sterilized by spraying with ethyl alcohol mixed with water (4%). The inoculum (spore suspensions of *Rhizoctonia solani* and *Fusarium pallidoroseum*) were prepared by mixing four days old culture by adding 4-5ml of water. The slants were gently tapped and shaken after which the suspension was collected with a syringe. Spraying was done to the base of the plants and the adjacent soil side by side control was maintained. Such inoculated plants were covered with polythene bags and kept for 4 days at temperature of 25 °C and 100% RH, after that the poly bags were removed and observation were taken regularly. After development of typical damping off symptoms in inoculated plants, the fungus was re-isolated. Its morphological characters were studied again and compared with that of the isolate obtained originally from the naturally infected plants.

The botanical and chemical fungicides were collected according to the local availability. Locally available plant species having antifungal and therapeutic properties were collected from various farms and gardens of Bhubaneswar. Ten number of locally available plant parts viz; rhizome of *Curcuma longa* and *Zinziber officinale, clove of Allium sativum, bulb of Allium cepa, and leaf of Azadirachta indica, Pongamia pinnata, Annona squamosa, Eucalyptus* sp., Lantana *camara and Chrysanthemum* sp. were taken. Nine numbers of fungicides @ 0.2% of Carbendazim 12% WP + Mancozeb 63% WP, Chlorothalonil 75% WP, Carboxin 37.5% + Thiram 37.5%, 0.15% of Thiophanate methyl 50% WP, and 0.1% of Tebuconazole 25.9% EL, Hexaconazole 5% SC, Difenoconazole 10% WP, Azoxystrobin 25% EC, Propiconazole 25% EC were taken to test their efficiency against the test pathogens. Fresh plant materials of different plant species were thoroughly cleaned, surface sterilized with 2% sodium hypochlorite and washed well with sterile water. The predetermined plant parts were ground along with sterile water at the rate of 1:1 w/v using pestle and mortar and the macerate was filtered through a Whatman No.1 filter paper under sterilized condition to get the clear plant extract (100%). The fungicides and extract of plant at 10% were tested against the pathogens by poisoned food technique (Thapliyal, 1971) [9].

Observations on radial mycelium growth/colony diameter of the pathogen were recorded at 24 hrs. interval and continual till the untreated control plate was fully covered with mycelia growth of the test pathogen. The Per cent mycelia growth inhibition of the test pathogen with the test botanicals/plant extract and fungicides over untreated control were calculated by applying the following formula (Vincent, 1927) [6].

The colony diameter was recorded and per cent inhibition of growth of the pathogens over control was estimated. The efficacy of plant extracts or botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by following formula.

$$\text{Percent Inhibition (I)} = \frac{C-T}{C} \times 100$$

Where,
C = Growth (mm) of test fungus in untreated control plate
T = Growth (mm) of test fungus in treated plates

Results and Discussion

The present studies on brinjal particularly on damping-off disease was carried out during 2019-2020 on various aspects like symptomatology, isolation, pathogenicity of the test pathogens, bioassay of fungicides and botanicals (*in-vitro*). The results obtained from various studies and experiments are described in following paragraphs.

The per cent disease incidence was calculated with the formula mentioned in the methods. As mentioned in the methods for each plot three patches of one square meter area were selected and the mean per cent disease incidence obtained from those patches was recorded. The results thus obtained are given here in the following Table 1.

Table 1: Percentage of disease incidence that were observed

Sl. No.	Details of the plot	Location	Percent disease incidence (%)
1	Nursery Plot of brinjal (Utkal Tarini)	All India Coordinated Research Project (AICRP) on Vegetable, OUAT, Bhubaneswar	*R. Solani* (4%)
			F. Pallidoroseum (5%)
2	Nursery Plot of brinjal (Arka nilanchalashyama)	Central Horticultural Experimental Station (CHES), Bhubaneswar	*R. Solani (5%)*

The collar region turned brown, shunken and sclerotial bodies were observed. The affected seedlings were toppled. These types of symptoms were observed in brinjal seedlings due to *R. Solani* and *F. Pallidoroseum* infection. Stem portion near soil show yellowing and rotting than seedlings became wilted. Infected seedlings were collected and thin section of infected parts were prepared and examined under microscope to observe the morphological characters of the fungus. From the fresh affected samples, the test pathogens were observed under microscope in brinjal. These were *R. Solani and F. Pallidoroseum*. The pure culture of these fungi was maintained in petriplates after the isolation of the fungus and the sub culturing was done in slants for *in-vitro* studies. Further the cultural behavior of the test pathogen was observed. The culture identification was done at Department of Plant Pathology, Indian Agricultural Research Institute (IARI) and New Delhi. The *Rhizoctonia solani* and *Fusarium pallidoroseum* were isolated from seedling of brinjal (Table 2).

Table 2: Details of identified strains

Sl. No.	ID No.	Source	Fungus
1	11,286.20	Brinjal seedling	*Rhizoctonia solani*
2	11,288.20	Brinjal seedling	*Fusarium pallidoroseum*

The pathogenicity of abovementioned fungi were proved by growing the seedlings on poly bags by flowing Koch's postulates. In brinjal pathogenicity of *R solani* and *F. pallidoroseum*. The symptoms of these pathogens were observed after 4 days of application of spore suspension to the corresponding plants whereas in control no symptoms were observed. The pathogens were again isolated from diseased seedlings of corresponding plants and compared with initial pathogens applied to the plants, where they were found to be same.

The fungicides, i.e. carbendazim, carboxin + thiram, mancozeb, hexaconazole and some locally available botanicals were successfully used in managing damping off disease of brinjal (Gholve *et al.*, 2014) [1].

The result related to readial growth and inhibition per cent of test fungi were presented in Table 3. Antifungal activities of various chemicals were assayed *in-vitro* by poison food technique and inhibition zone technique for *Rhizoctonia solani*, and *Fusarium pallidoroseum*, respectively. Results revealed that all the systemic fungicides were capable of inhibiting growth of test fungus at recommended dosage as compared to check (Vir and Hooda, 1989) [7].

Table 3: Bioassay of fungicides against the test fungi

Treatments	Organisms			
	Fusarium pallidoroseum		*Rhizoctonia solani*	
	Radial growth (mm)	Inhibition (%)	Radial growth (mm)	Inhibition (%)
Carbendazim +Mancozeb	00.8[a]	100.0 (90.0)*	00.9[a]	90.3 (71.8)*
Chlorothalonil	82.6[d]	13.7 (21.8)	80.0[d]	10.7 (19.1)
Thiophanate methyl	40.1[c]	52.8 (46.6)	40.3[c]	55.0 (47.8)
Tebuconazole	00.0[a]	100.0 (90.0)	00.0[a]	100.0 (90.0)
Hexaconazole	00.0[a]	100.0 (90.0)	00.0[a]	100.0 (90.0)
Difenoconazole	00.0[a]	100.0 (90.0)	00.0[a]	92.9 (74.5)
Azoxystrobin	12.2[b]	84.3 (66.7)	11.3[b]	87.3 (69.1)
Propiconazole	00.0[a]	100.0 (90.0)	00.0[a]	87.7 (69.4)
Carboxin +Thiram	00.0[a]	100.0 (90.0)	00.0[a]	100.0 (90.0)
Control	89.5[e]	0.0 (0.0)	89.7[e]	0.0 (0.0)

* Figures in the parenthesis are sin transformed value

NB: Similar superscripted alphabets are not significantly different at $p = 0.05$

Mixed fungicides Carbendazim + Mancozeb at 0.2%, Carboxin+ Thiram at 0.2% and Triazole group of fungicides viz; Tebuconazole, Hexaconazole, Difenoconazole and Propiconazole recorded inhibition of *Rhizoctonia solani* growth by 100% followed by Azoxystrobin of 0.1% (87.26%), and Thiophanate methyl at 0.15% concentration (57.96%). Least antifungal property was shown by Chlorothalonil at 0.2% (10.73%). Against *Fusarium pallidoroseum* maximum antifungal activity was shown by mixed fungicides Carbendazim + Mancozeb at 0.2%, Carboxin + Thiram at 0.2% and triazole group of fungicides *viz;* Tebuconazole, Hexaconazole, Difenoconazole and Propiconazole, recorded inhibition was 100% followed by Azoxystrobin of 0.1% (84.33%), and Thiophanate methyl at 0.15% concentration (52.84%). Least antifungal property was shown by Chlorothalonil at 0.2% (13.73%). The mixture of fungicides, i.e., Carbendazim+ Mancozeb at 0.2%, Carboxin + Thiram at 0.2% and Triazole group of fungicides viz., Tebuconazole, Hexaconazole, Difenoconazole and Propiconazole, recorded inhibition was 100% followed by Azoxystrobin of 0.1% (84.33%), and Thiophanate methyl at 0.15% concentration (52.84%). Confirming the above results Satija and Hooda (1987) [4] demonstrated that Dithane M-45 and Thiram were the best treatments for chilli damping-off disease. Irrespective to the above studies. The *R. solani* was inhibited completely when it was treated with leaf extract of *Azadirachta indica* next by *Allium sativum* (52.70%) followed by *Curcuma longa* (41.53%), *Lantana camara* (41.12%), *Annona squamosa* (30.06%), *Eucalyptus* sp. (25.93%), *Pongamia piñata* (19.23%), *Zingiber officinale* (18.97%) and *Chrysanthemum* sp. (5.3%) at 10% concentration. *Chrysanthemum* sp. at both the concentration was least effective in reducing fungal growth.

Bioassay of plant extracts on growth of *Rhizoctonia solani*, and *Fusarium pallidoroseum* were tested *in-vitro* at 10% concentration. All the plant extracts were significantly effective in reducing growth of *Rhizoctonia solani*, and *Fusarium pallidoroseum* as compared to control (Table 4). The growth of F. *pallidoroseum* and *R. solani* were completely stopped by applying 10% *Azadirachta indica.* Inhibition of growth of *R. solani* ranged from 0.0 cm to 88.7 cm against 90 cm in control, and of *F. pallidoroseum* ranged from 0.0 cm to 88.66 cm against 90 cm in control. The interaction between plant extracts and concentrations was found significant.

All plant extracts were increasingly effective in reducing mycelia growth with increase in concentration. Irrespective of concentration, *Azadirachta indica* was proved to be effective botanical and recorded maximum reduction of growth of the pathogens *R. solani*, and *F. pallidoroseum* by 100% which was significantly superior to all other plant extracts. The next best treatment against *R. solani* was *Allium sativum* (52.70%) followed by *Curcuma longa* (41.53%), *lantana camara* (41.12%), *Annona squamosa* (30.06%), *Eucalyptus* sp. (25.93%), *Pongamia piñata* (19.23%), *Zingiber officinale* (18.97%) and *Chrysanthemum* sp. (5.3%) at 10% concentration. Chrysanthemum at both the concentration was least effective in reducing fungal growth. Whereas for *F. pallidoroseum* after *Azadirachta indica*, *Allium sativum* (52.70%) followed by *Eucalyptus* sp. (41.53%), *Annona squamosa* (41.12%), *Lantana camara* (30.06%), *Curcuma longa* (25.94%), *Allium cepa* (21.92%), *Pongamia piñata* (19.28%), *Zingiber officinale* (17.99%) and *Chrysanthemum* sp. (4.03%) at 10% concentration. *Chrysanthemum* sp. at both

the concentration was least effective in reducing fungal growth. Whereas for *F. pallidoroseum* after *Azadirachta indica*, *Allium sativum* which inhibited control 52.70% followed by *Eucalyptus* sp. (41.53%), *Annona squamosa* (41.12%), *Lantana camara* (30.06%), *Curcuma longa* (25.94%), *Alium cepa* (21.92%), *Pongamia piñata* (19.28%), *Zingiber officinale* (17.99%) and *Chrysanthemum* sp. (4.03%) at 10% concentration. Similar findings have been reported by Islam and Faruq (2012) [3] who evaluated the seed treatment with neem leaf (*Azadirachta indica*), garlic clove (*Allium sativum*), ginger rhizome (*Zingiber officinale*), bel leaf (*Aegle marmelos*), turmeric rhizome (*Curcuma longa*), and onion bulb (*Allium cepa*) against damping-off, seed germination and growth characters of tomato (*Lycopersicon esculentum* L.), eggplant (*Solanum melongena* L.) and chilli (*Capsicum annum*) seedlings. He concluded that the most effective against seedling damping off was neem leaf extract followed by garlic clove and allamonda (*Allamanda cathartica*). The highest seed germination of tomato seeds (86.67%) was observed under neem leaf extract effect.

Table 4: Bioassay of botanicals against the test fungi

Treatments	Organisms			
	Fusarium pallidoroseum		***Rhizoctonia solani***	
	Radial growth (mm)	**Inhibition (%)**	**Radial growth (mm)**	**Inhibition (%)**
Curcuma longa	72.7^{de}	19.2 (25.94)*	50.3^{c}	44.0 (41.5)*
Zinziber officinale	81.3e	9.6 (17.99)	80.3^{e}	10.7 (19.0)
Allium sativum	33.0^{b}	63.3 (52.70)	33.0^{b}	63.3 (52.7)
Allium cepa	77.3^{e}	14.0 (21.92)	87.3^{e}	2.8 (7.8)
Azadirachta indica	00.0^{a}	100.0 (90.00)	00.0^{a}	100.0 (90.0)
Pongamia pinnata	80.0^{e}	11.1 (19.28)	80.0^{e}	11.1 (19.3)
Annona squamosa	51.0^{c}	43.3 (41.53)	67.3^{d}	25.1 (30.1)
Eucalyptus sp.	50.3^{c}	44.0 (41.53)	72.7^{de}	19.2 (25.9)
Lantana camara	67.3^{d}	25.1 (30.06)	51.0^{c}	43.3 (41.1)
Chrysanthemum sp.	88.7^{f}	1.5 (4.03)	87.7^{e}	2.6 (5.3)
Control	90.0^{f}	0.0 (0.00)	90.0^{fe}	0.0 s(0.0)

* Figures in the parenthesis are sin transformed value

NB: Similar superscripted alphabets are not significantly different at $p = 0.05$

It was observed that in brinjal, *R* Solani and *F Pallidoroseum*, a were the cause of the damping off of disease in nursery. Mixed fungicides Carbendazim + Mancozeb at 0.2%, Carboxin + Thiram at 0.2% and Triazole group of fungicides *viz*; Tebuconazole, Hexaconazole, Difenoconazole and Propiconazole recorded inhibition of *Rhizoctonia solani* growth by 100% followed by Azoxystrobin of 0.1% (87.26%), and Thiophanate methyl at 0.15% concentration (57.96%). Least antifungal property was shown by Chlorothalonil at 0.2% (10.73%). Mixed fungicide Carbendazim + Mancozeb, Carboxin + Thiram showed 26.96% or 21.90% inhibitions which were at par with each other and least inhibition of growth was shown by Chlorothalonil (6.75%). Tebuconazole at 0.1% was the most effective fungicide and Chlorothalonil was the least effective fungicide against the test fungi *Rhizoctonia solani*, *Phytophthora* sp. and *Fusarium pallidoroseum*. Bio efficacy of plant extracts on growth of *Rhizoctonia solani*, and *Fusarium pallidoroseum* were tested *in-vitro* at 10% concentration. All the plant extracts at both the concentrations were significantly effective in reducing growth of *Rhizoctonia solani* and *Fusarium pallidoroseum* as compared to control. The inhibition of growth of *R. solani* ranged from 0.0 cm to 88.66 cm against 90 cm in control, and of *F. pallidoroseum* ranged from 0.0 cm to 88.66 cm against 90 cm in control.

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