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Research Article

Fungicidal Activity of Aqueous Leaf Extract on *Alternaria brassicae.*

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ABSTRACT

Alternaria brassicae is a widespread pathogen causing black leaf spot in the common cabbage (*Brassica oleracea* Linn.). Ample spectrum of effective chemical fungicides is in use against this pathogen. The agricultural areas of the Nilgiris are fragmented shola forests of this biodiversity hot spot. The indiscriminate usage of chemical fungicides may cause physiological damage, including the endocrine disruption not only in the human but in wild life also. So a universally available bio-fungicide is in quest. With this view the study was conducted to find a biofungicide against the common crucifer pathogen *Alternaria brassica*. The experiment tested 10 plant extracts against the pathogen in culture plates. The pathogen is collected from the field and the pure line was isolated, the pathogenesis was confirmed by re-infecting the pathogen on host under controlled condition. The majority of the extracts though show efficient in controlling the pathogen, *Solanum nigrum* is more effective and the activity is almost found equal to chemical fungicides. The results were discussed in relation to the literature and steroidal saponins were concluded to be the active principle in controlling the pathogen.

Key words: Bio-fungicide, *Solanum nigrum*, Steroidal saponins, *Alternaria brassicae*, Colony formation, Nilgiri Biosphere.

INTRODUCTION

Fostering of exotic vegetables like, potato, cauliflower and cabbage forms the major economy of Nilgiris. *Brassica oleracea* Linn. (Cabbage), alone is cultivated in approximately of 2129 acres of land, amongst these are assorted exotic species, both as irrigated and rain fed. Due to the increasing habitual demand, its cultivation is increasing swift and the farmers are gradually adopting *Brassica oleracea* as a main cash crop. The Nilgiris with temperate climate in the tropical region has mild winter, mild summer, rich soil conditions and different elevations making the crop vulnerable to lot of pathogens. *Alternaria brassicae* is a widespread pathogen not only to the cabbage but also to field mustard (*Brassica campestris)*, leaf or Chinese mustard (*Brassica juncea*, broccoli, cauliflower (Brassica oleracea var. botrytis), cabbage, Chinese or celery cabbage (*Brassica pekinensis*), turnip (*Brassica rapa*), radish (*Raphanus sativus*). To control this commonly occurring fungus countless chemical fungicides are available[1].

The intensive crop cultivation demands the utilization of strong chemical fungicides, which are not only expensive but also hazardous for all living beings. Usage of bio-control methods is the solitary alternate solution that can reduce toxic chemical substances. The searches for a harmless fungicide that do not have an ecological impact and can be involved in sustainable agriculture are inquest[2]. Several higher plants and their constituents have shown success in controlling plant disease, and are proved to be harmless and non-phytotoxic unlike chemical fungicides[3]. Several leaf extracts like, *Eucalyptus, citriodora*[3], *Lantana camara*[4] *Ocimum basilium*[5] and *Azadirachta indica*[6] can be efficient in controlling agents against *Alternaria brassicae*. The plants like *Solanum nigrum*[7] *Azardirachta indica*[6] are proved to contain secondary metabolites at high concentration. Glycoalkaloids and saponins[8] are reported to be present in very high concentration in solanum species. The alkaloids in these plants have established to have antifungal properties[9,10]. The current study aims to screen the effect of various plant extract against the common cabbage fungus *Alternaria brassicae.*

MATERIALS AND METHODS

The study was conducted during the rainy season (June to August) at the Ootacamund, the Nilgiris, Tamil Nadu, India. Cabbage leaves infected with *Alternaria brassicae* (plate1) were collected from various agricultural fields of Ooty. Infected leaves were cut into small bits and surface sterilized with mercuric chloride. Sterilized inoculum was applied into PDA (Potato Dextrose Agar) medium for mycelia growth. The colonies were developed and pure lines were sub cultured from the sporulating colonies

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Plate 1: Infected cabbage leaves

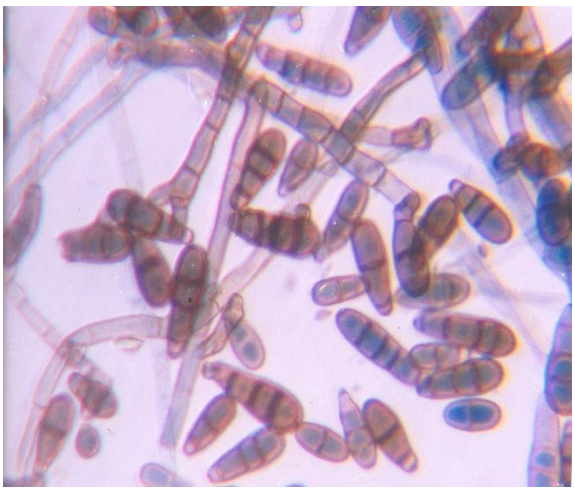

Plate 2: Spores of Alternaria brassicae

Table 1. Effect of fungicides and plant extracts on colony formation

	Colony Diameter (Mean ± SD) mm		
Name of the extract	1% (w/v)	2% (w/v)	3% (w/v)
Control	43.67 ± 1.86	43.00 ± 1.41	40.00 ± 0.89
Mancozeb#	15.50 ± 1.52	08.00 ± 1.41	05.67 ± 2.07
Metalaxyl #	10.67 ± 1.21	07.00 ± 2.67	05.33 ± 2.07
Coriandrum sativum	39.16 ± 1.70	29.50 ± 1.05	24.17 ± 1.70
Mentha pipera	33.33 ± 1.21	33.05 ± 1.05	31.67 ± 0.89
Eucalyptus globules	28.67 ± 1.03	25.17 ± 0.75	21.33 ± 0.85
Azaradichta indica	30.67 ± 0.82	28.17 ± 1.17	21.50 ± 1.05
*Moringa oleifera**	39.66 ± 0.82	36.67 ± 1.86	30.50 ± 2.74
Murraya koenigi	35.17 ± 2.93	31.33 ± 2.42	27.67 ± 2.07
Solanum nigrum#	09.67 ± 1.33	09.00 ± 0.94	08.00 ± 1.26
*Centella asiatica**	38.33 ± 2.42	35.00 ± 1.79	31.50 ± 2.26
Leucas aspera	30.67 ± 2.42	30.00 ± 2.28	28.50 ± 1.64
Ruta graveolens	27.50 ± 1.87	24.00 ± 1.41	23.33 ± 2.16

**Non significant with Control (P<0.05) # Significant P<0.05 with other extracts*

(Plate 2)[11]. The colonies were identified[12] and the pathogenesis was confirmed by reapplying the pure line spores in cabbage plants (Plate 3) under controlled conditions in the lab. Fresh leaves of *Coriandrum sativum*, *Mentha piperata*, *Eucalyptus globules*, *Azaradichta indica*, *Moringa oleifera*, *Murraya koenigi*, *Solanum nigrum*, *Centella asiatica*, *Ruta graveolens*, *Leucas* aspera

Plate 3: Re-infected plants and control to check the pathogencity of pure line

were collected and 1%, 2% & 3% (w/v) slurry were prepared in
distilled water using mechanical homogenizer. The extract was further sonicated for 5 minutes to make perfect extraction. Each of this leaf slurry was mixed with the PDA medium at 1%, 2% and 3% concentrations without altering the constituent composition. Negative control included sterile distilled water instead of extracts and positive controls were prepared using various known fungicides like Mancozeb (Dithane) and Metalaxyl (Ridomil) at same concentrations mentioned above. All the plates were incubated at 28oC for 14 days until the colonies were developed. The colony diameter was measured using graph paper. The average of six colonies was counted in each case and Arithmetic mean ± standard deviations were calculated. The statistical analysis was performed by using the two-way ANOVA and inter-group comparison was calculated using Newman-Keuls Multiple Comparison method.

RESULT

The pathological studies on *Brassica oleracea* inoculated with the isolate of *Alternaria brassicae* showed the characteristic disease symptoms (sickly appearance with grey spot on the leaves). From the diseased plant the pathogen was isolated and it was compared with the original culture and was found to be identical (data not and various extracts on the growth and sporulation of

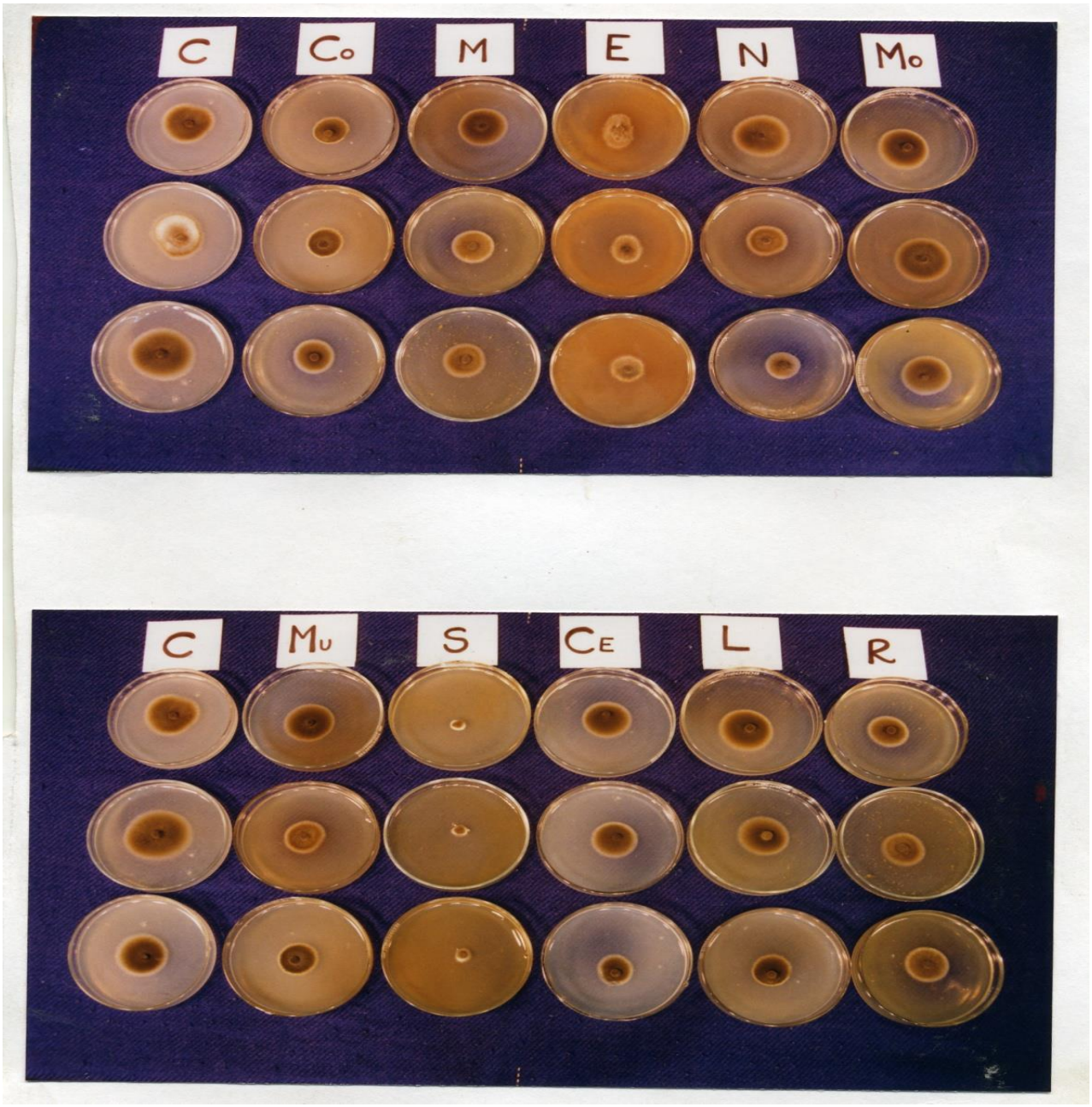

Plate 4: The activity of various extracts.
C: Control, M: Mentha piperata N: Azaradichta indica Co: Coriandrum sativum E: Eucalyptus globules Mo: Moringa oleifera Mu: Murraya koenigi L: Leucas aspera Ce: Centella asiatica S: Solanum nigrum R: Ruta graveolens

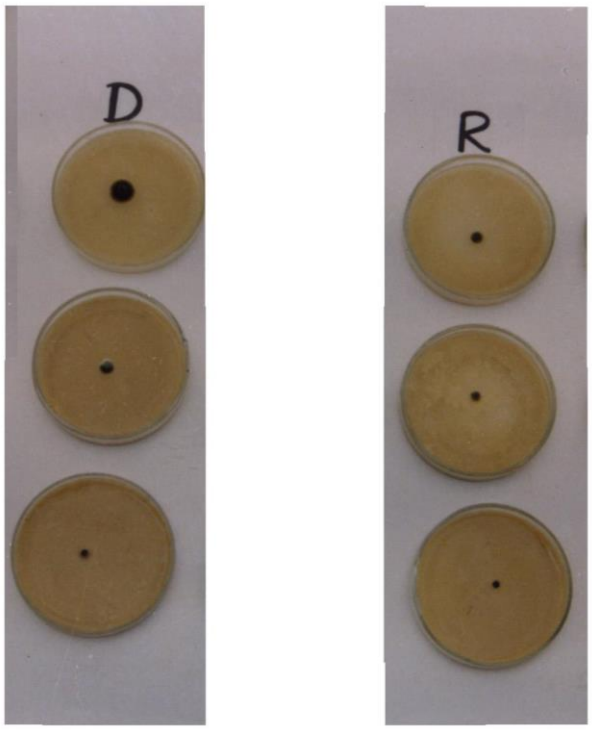

Plate 5 Activity of Positive Controls
D: Dithane R: Ridomil

shown). Table I shows the effect of chemical fungicides Alternaria brassicae. Statistical analysis indicates that the chemical fungicides (the positive controls) and the *Solanum extract* belong to a homogenous population. This clearly illustrates the *Solanum extract* have antifungal activity approximating that of chemical fungicides (Plate 4 & 5). Except *Moringa* and *Centella*, all the other plant extracts shows a considerable inhibitory effect on fungal colony formation. However, the activity of all the effective extracts other than *Solanum* is statistically parallel proving that these should not to be considered as alternate for chemical fungicide. Nevertheless, the concentration study depicts the increasing concentration have no effect in controlling the fungus.

DISCUSSION

Use of plant products for the management of phyto-pathogenic fungi is swift fetching an important module of Integrated Disease Management (I.D.M.) programme. The natural plant products are bio-degradable and thus eco-friendly, are catching the concentration of the scientists worldwide. Such products from higher plants are relatively broad spectrum, bio-efficacious, economical and environmentally safe[3]. Cabbage is an economically valuable crop not only for the farmers of the Nilgiri Biopshere Reserve (NBR) but worldwide. The arbitrary usage of the common fungicides has made the man and wild life susceptible to a wide array of diseases. As an alternate, many plant products are in search. Plants contain various kinds of phytochemicals like, Saponins, Alkaloids, Flavanoids etc., (commonly called secondary metabolites) are bringing the antimicrobial effects. In the current study it is well proved that all most all plants used in the study have at least little effect in controlling the fungus. Yet, it is clearly shown that except *Solanum nigrum* no other extract have the similar effect of chemical fungicide (positive controls). The study was aimed to find an ample plant product which can be used as a substitute of commercially available chemical fungicide. The result shows that among a group of plants studied; only *Solanum nigrum* is efficient in this aspect. S. nigrum grow abundantly in the Nilgiris in wild and in open fields and is reported to have medicinal properties[13]. As per the results obtained the slurry itself can be used in the field as a plant product surrogate to control the disease causing pathogen *Alternaria*. The effect of *Solanum nigrum* in controlling the pathogen *Alternaria brassicola* is reported by Muto etal[14]. The results of the current study show this is applicable in case of the *A.brassica* also. *Solanum nigrum* contain secondary metabolites like steroidal saponins[8]. The steroidal saponis are reported to be give protection for plants against various pathogens[15]. Six new steroidal saponins are reported from the *solanum nigrum* other than the known degalactotigonin[8]. Steroidal saponins are reported to have antifungal activity against a variety of fungus like Candida and *Aspergillus*[16]. In the current study also saponins may be the active principle responsible for the fungicidal activity. This gains support from the observations of[17,18]. However, the activity of these compounds from *solanum* and its fractions against *Alternaria* is still obscure and need more research. The development of such an active principle against *Alternatia* will be promising towards the quest of a natural fungicide. However, the current research clearly shows that the even the usage of 1% slurry of the *Solanum nigrum* extract itself can control the disease in the field and is economical without any side effects. The present report is the foremost one screening the activity of *Solanum nigrum* extract against the *Alternaria*. Further research is desired in this aspect in order to explicate the molecular mechanism of control and to reveal the component of a Solanum accountable for controlling *Alternaria*. The research furthermore suggests the want of extensive work in this field of plant pathology so to find the effectiveness of the active principle in the *Solanum nigrum* against other pathogenic microorganisms also. The research is unique and gains prominent consideration as a development of a commercial "phyto-fungicide" substitute against the chemical fungicides like *Mancozeb (Dithane) and Metalaxyl (Ridomil).*

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