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# Seasonal occurence of Fungal Diversity in Castor Plant (*Ricinus communis* L.): The Primary Food Plant of Eri Silkworm [*Samia ricini* (Denovan)]

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## Abstract

*Ricinus communis L. is a primary food plant of eri silkworm [Samia ricini (Danovan)], a domesticated polyphagous multivoltine lepidopteran insect which is responsible for producing sericin. It is also a non-edible oilseed crop plant with unique oil compositions for the chemical industry as well as an important source of income for the people of North East India. Fungal diversity of castor was studied from the infected tender and mature leaves during two seasons i.e. summer (March-June) and winter (November-February) along with the meteorological parameters. A total of 11 fungal species were isolated during the seasons. The present investigation showed that 8 species of fungi viz., Alternaria ricini, Aspergillus fumigatus, Cercospora ricinela, Curvularia clavata, Fusarium sp. were dominant during the summer and 3 fungal species viz., Emericella nidulans, Leveillula taurica, Melampsora ricini were restricted to the winter season only. Maximum numbers of fungal species were isolated during the summer season as compared to winter season. Positive correlation is observed between the temperature and fungal colony in summer and rainfall and fungal colony in winter season. Whereas, negative correlation is observed between relative humidity and assemblage of fungal colonies and temperature and fungal colonies in winter season and rainfall and fungal colonies in summer seasons. A. ricini, Penicillium sp., Fusarium sp. and C. ricinela were found to be most abundant species during all the seasons. Among these fungal species some are infectious towards the castor leaf which may lower the quality and quantity of the leave production as well as growth and development of the plant.*

**Keywords**: Fungal diversity, *Ricinus communis,* season, Eri silkworm.

## Introduction

Castor (*Ricinus communis* L), the major food plant of eri silkworm [*Samia ricini (*Danovan)), grows abundantly in North Eastern region of India (latitudes, 21°59´N 29°40´N; longitudes, 89°51´E 97°25´E) [1]. The environmental factors of the area are suitable for growth of the plants as well as various flora and fauna long with insect pathogens[2]. Eri silkworm [*Samia ricini* (Danovan)] belongs to the family Saturniidae, which is completely domesticated polyphagous multivoltine silkworm among the vanya silkworms in North Eastern region of India. Eri culture plays significant role in rural livelihood security especially among marginalized and weaker section of the society. Eri culture is prevalent mainly amongst the tribal area in hill districts of Assam, Nagaland and northern hill areas of Meghalaya. Lower Brahmaputra Valley is the traditional homeland of eri spinners and weavers producing bulk of eri yarn and fabric. Of late, eri culture has been introduced to many non-traditional states of India. Approximately, 1.3 lakh families with plantation area of 26000 hectares are involved in eri culture in northeastern region of the country[3].

Castor belongs to the family Euphorbiaceae is a semi-tropical perennial grown extensively in warm temperature and tropical regions of the world[4]. The caster plant is a robust annual plant that grows between 2 - 5 meters in one season with a temperature of about 23°C and relative humidity of about 50%. Castor is a drought resistant crop prefers 380 – 500 mm rainfall during growing season of 140 – 150 days. It does not tolerate heavy rainfall or water logging. It prefers deep sandy loam soil (pH 6)[4,5]. Under dry conditions, yields are about 1.0 - 1.2 t/ha but reaches 1.5-1.8 t/ha under irrigation. Castor oil is known as one of the best laxative and purgative. Castor oil is used for a range of industrial purposes from soap making to vanishes and also used very effectively in the treatment of rheumatic and skin disorders[6]. The surface of leaves contain stimulatory or inhibitory substances that regulate the colonization of leaf surface organisms[7]. It is one of the major microbial habitat which provides shelter to diverse microbial organisms like bacteria, fungi, yeast, protozoa etc[8]. The phylloplane microflora is subjected to the influence of various environmental factors and physiological changes in the plants and that too due to onset of diseases[9]. The phylloplane microorganisms also have positive, negative or neutral influence on the host plant[10]. In addition, these microorganisms involve in carbon and nitrogen cycle[11] which lead to stabilized the ecosystem by decomposing the organic matter[12]. Temperature, rainfall and relative humidity are the most important environmental factors which inspire the pathogen for infection and disease development[13]. The growth stages and forms of host plants also influenced the disease occurrence in the plylloplane. Epidemiological data, therefore, are essential to study the disease incidence in the host plant as well as to develop disease management strategies[14].

The knowledge of phylloplane micro-organisms of infected leaf has been important to determine the foliar diseases. Many microorganisms are capable of influencing the growth of the pathogens[15]. In the present study quantification of the phylloplane microorganisms of infected castor was attempted during summer and winter season. Mycoflora of castor varies in size and diversity depending on the influence of numerous biotic and abiotic factors which affect their growth and survival. These factors also include temperature and humidity[16]. The major groups of leaf surface mycoflora are present at any time of the year, but there are also evidences for seasonal succession[17]. The succession of mycoflora on leaf surface presents an interesting model for studying functional relationships between plants and mycoflora[18]. The current investigation is one of the series of studies concerning the ecological relationships and interactions of fungi in the phylloplane of *R. communis.* The aim of this study is to determine the main constituents' of the mycoflora on the infected leaves of *R. communis* during different seasons.

## Material and Methods

**Sample collection and isolation:** The experiments were conducted in Central Muga Eri Research and training Institute (CMER and TI), Jorhat, Assam (India). Both infected tender and mature leaves of castor showing typical symptoms were collected from Chenijan Germplasm field during winter and summer season (figure-1). The samples were collected in the sterile polythene bags for the isolation of microflora. Both the infected leaves were cut into small pieces (1 $cm^2$) and placed separately in 100 ml sterilized distilled water in 250 ml Erlenmeyer flasks and were shaken for 20 min on a shaker (120 rpm) and the suspension was treated as stock and followed by serial dilution up to $10^{-7.}$ Then 100 µl of each dilution was transferred to petriplates containing 20 ml Potato Dextose agar media (Himedia Ltd., Mumbai) and incubated at 25±2°C in BOD incubator for a period of three days[19]. Each treatment contains three replications and observation of different fungi colonies was made after 4 days of incubation and counts the colony numbers in a colony counter. During this period monthly meteorological data viz. temperature, relative humidity and rainfall were also recorded.

**Characterization and Identification**: Fungal colonies were isolated after 72 hrs of incubation and the culture were further purified by single hyphal tip method and maintaind on Czepecks Dox Agar (Himedia Ltd., Mumbai) slant for further use. Identification of different fungal colonies was done based on colony characteristics and spore morphology as per method and the keys described in the Manual of Microbiological Methods (Society of American Bacteriologist) and standard methods[20-23]. Fungal isolates were identified on the basis of cultural, morphological and microscopic characteristics. The mycelial and spore characters of the fungi were studied under phase Contrast microscope (Leica, Germany) with higher magnifaction.


**Figure-1**
**Infected mature(a) and tender (b)leaf of castor**

**Statistical analysis:** The Statistical analysis of the data and graph were done using the Microsoft Excel Programme and the correlation coefficient and significance were carried out by statistical software programme 'SPSS version 11'. Least significance difference was calculated to compare the significant differences between individuals.

## Results and Discussion

In the present study 11 fungal species were recorded from different groups in two different seasons i.e. winter and summer. The fungal isolates were mainly belongs to the class Ascomycetes, Deteromycetes and Oomycetes. The study also reveals that the maximum numbers of fungal colonies were isolated during summer as compared to winter. *Alternaria ricini, Aspergillus fumigates, Curvularia ricinela, C. clavata, Fusarium* sp., *F. moniliform, Penicillium parasitca* and *Penicillium* sp. were dominant in the summer season. On the other hand, *Emericella nidulans, Leveillula taurica, Melampsora ricini* were mainly found in the winter season (figure 3). As shown in table-1, the average fungal population (cfu/$cm^{2)}$ was more in mature leaf (104.4) during summer then mature leaf (81.8) during winter followed by summer tender leaf (28.7) and winter tender leaf (20.2) respectively. Positive correlation was observed during between the temperature and fungal colonies in summer season and between rainfall and fungal colonies in winter season. Whereas negative correlation was observed between relative humidity and assemblage of fungal colonies and between temperature and fungal colonies in winter season and rainfall and fungal colonies in summer seasons (table 2).

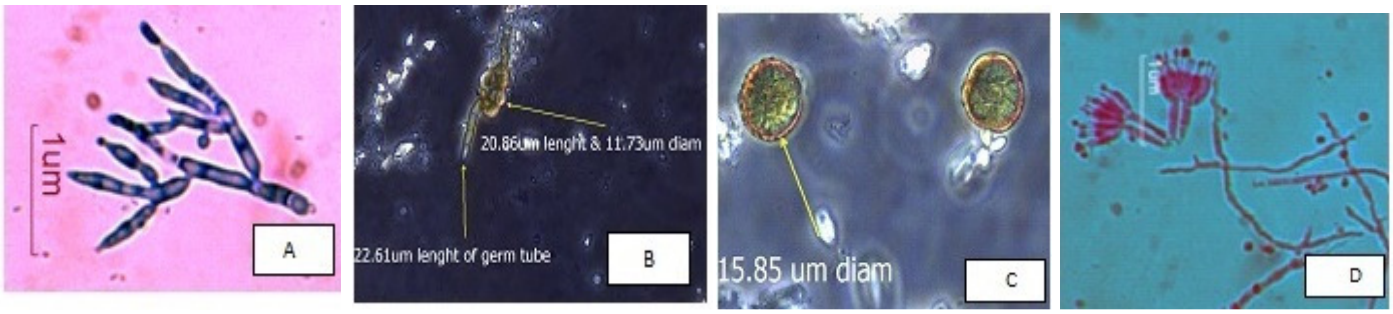


**Figure-2**
**Fungal spore and conidiophores (A - *Fusarium* sp.; B -*Alternaria* sp.; C- *Phytopthora* sp.; D-*Penicillium* sp.)**

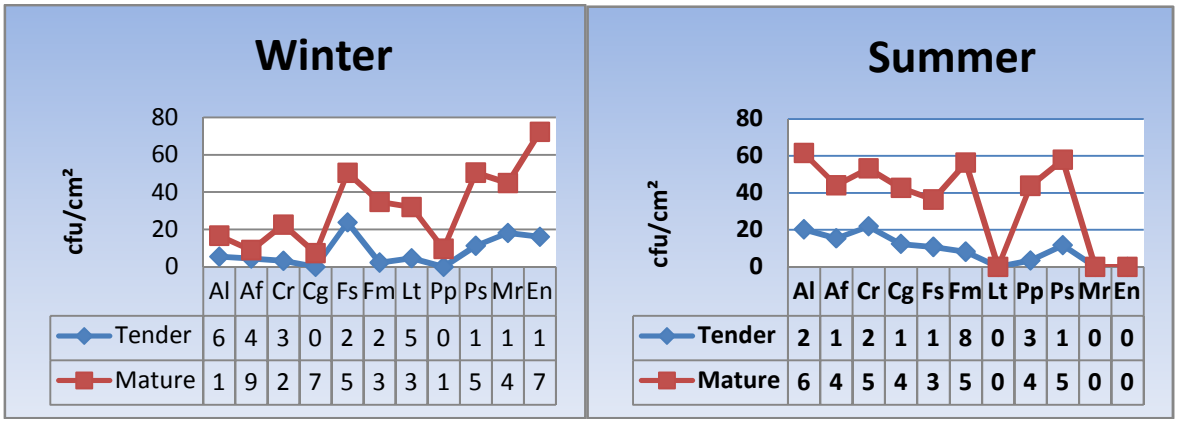


**Figure-3**
**Seasonal occurrence of different fungal species (CFU/cm2) in the infected tender and mature leaves of castor**
*[Al=Alternaria ricini, Af= Aspergillus fumigates, Cr= Cercospora ricini,Cg= Curvularia clavata ,Fs=Fusarium sp.,Fm=Fusarium moniliform,Lt= Leveillula taurica,Pp=Phytopthora parasitica,Ps=Penicillium sp.,Mr= Melampsora ricini ,En= Emericella nidulans,]*

**Table-1**
**Meteorological parameters and total number of fungal colonies at different categories of leaves during different seasons**

| Season | Temp(0°) | RH (%) | Rainfall(mm) | Fungal colony in different leaves cfu/cm$^2$ | |
|---|---|---|---|---|---|
| | | | | Tender | Mature |
| Summer | 26.025 | 87.98 | 108.8 | 28.7 (±10.1) | 104.4(±22.9) |
| Winter | | | | 20.2(±6.6) | 81.8(±17.2) |

**Table-2**
**Correlation coefficient between temperature, humidity, rainfall and fungal population on *R. communis* leaves**

| Parameter | Correlation /Significance | Fungal colony in winter | | Fungal colony in summer | |
|---|---|---|---|---|---|
| | | Tender | Mature | Tender | Mature |
| Temperature (°C) | Correlation | -0.400 | -0.492 | 0.893 | 0.966* |
| | Significance (Level:0.01) | 0.600 | 0.508 | 0.107 | 0.034 |
| Humidity (%) | Correlation | -0.991*** | -0.941 | -0.042 | 0.181 |
| | Significance (Level:0.01) | 0.009 | 0.059 | 0.958 | 0.819 |
| Rainfall (mm) | Correlation | 0.969* | 0.924 | -0.090 | -0.320 |
| | Significance (Level:0.01) | 0.310 | 0.076 | 0.910 | 0.680 |

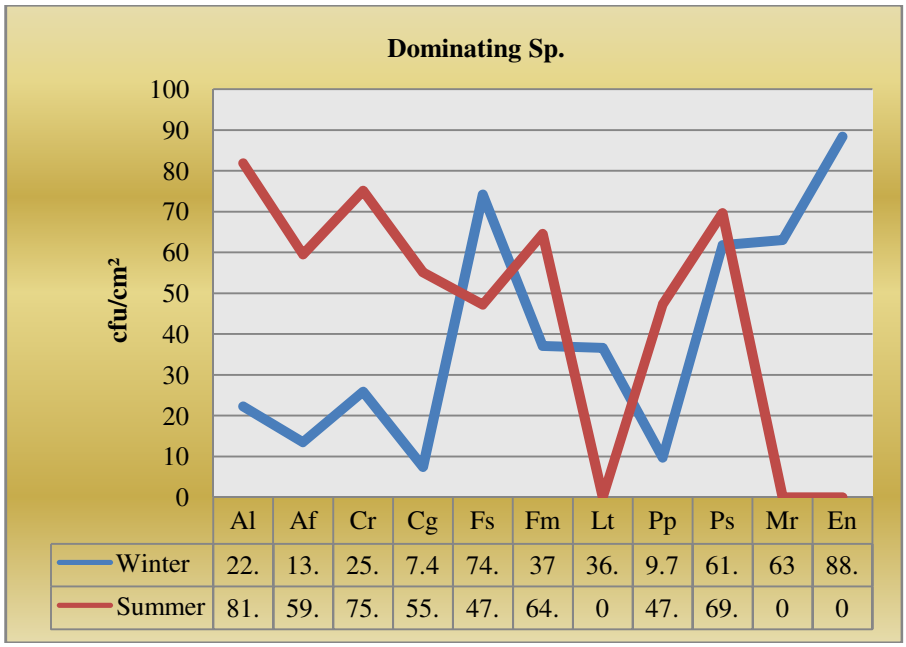


**Figure-4**
**Dominating species from average of tender and mature leave during both the seasons**

In mature infected leaves, *A. ricini* (61.5 cfu/cm$^2$) was found to be the most abundant species during the summer followed by *Penicillium sp.* (57.9 cfu/cm$^2$), *F. moniliform* (56.3 cfu/cm$^2$) and *C. ricinela* (53.2 cfu/cm$^2$) respectively as compared to the tender leaves. During the winter season, *E. nidulans* (72.2 cfu/cm$^2$) was found to be the most abundant, followed by *Penicillium sp.* (50.4 cfu/cm$^2$) and *Fusarium* sp. (50.3 cfu/cm$^2$) in mature leaves. But in tender leaf, *Fusarium sp.* (23.8 cfu/cm$^2$) followed by *Melampsora ricini* (18.2 cfu/cm$^2$) and *Penicillium sp.* (11.3cfu/cm$^2$) (figure-3).

Among the dominating species in the tender and mature leave, *A. ricini* (81.8 cfu/cm$^2$) was dominated followed by *C. ricinela* (75.1 cfu/cm$^2$) and *Penicillium sp.* (69.6 cfu/cm$^2$). In summer, *E. nidulans* (88.3cfu/cm$^2$) was found to be dominating in winter followed by *Fusarium sp.* (74.1cfu/cm$^2$) and *M. ricini* (63 cfu/cm$^2$).

The study reveals that the most abundant species i.e. *A. ricini, Fusarium sp.* and *Penicillium sp.* were frequently distributed throughout the year but it showed higher abundance in summer than winter possibly due to suitable optimal temperature, humidity, rainy weather and greater soluble nutrient availability. Khara and Singh have also studied the seasonal fluctuation and behaviour of fungi on leaves in relation to meteorological factors and found similar results[24]. The presence of maximum number of fungi in summer season may be due to greater multiplication of micro-organism with the availability of sufficient nutrition and other suitable environmental factors in the phylloplane[6,23]. Carbon and Nitrogen in fungal cell are the most important component which have vital role in structural framework and protein synthesis[25].

## Conclusion

The study reveals that the number and abundance of mycoflora isolated from the infected leaves of *R. communis* varied according to seasonal changes in meteorological and availability of nutrition. *A. ricini was* predominant between all isolated fungi. Further, pathogenic studies of this fungus will also be a great importance because among these species *A. ricini* is responsible for *Alternaria* blight of castor, *P. parasitica* for seedling blight, *L. taurica* for powdery mildew and *C. ricinella* for leaf spot disease and *M. ricini* for leaf rust disease[26]. *C. lunata* is the major disease of garlic bulbs[27] and some aspergillus and fusarium species secrete aflatoxin which may also pathogenic to humen and animal beings[28]. The systematic studies will lead to the illustration of identification characters of pathogenic fungus occurring in castor ecosystem. The systematic characters will help to develop diagnostic keys supplemented with information on symptoms of diseases, its extent of damage, life cycle, and distribution and management strategies. Quantitative knowledge of these surveys could be usefull in development of disease forecasting and forewarning systems and for improving control measures of fungal diseases through integrated approach.

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**Table-3**
**Morphological and Cultural characteristics of fungal strains isolated from infected leaf of of castor**

| Isolate | class | Cultural characteristics | | | | | Microscopic observation | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Colour | Margin | Reverse | Growth | Texture | Mycelium | Conidiophore | Conidia& size |
| *Alternaria ricini* | Deuteromycetes | Grey | Irregular | Blackish | Fast | Powdery | Septate & branched | Erect,sort & branched | Multicelled, obclavate with a short conical beak.Smooth walled and pale brown in colour.size:39.77 X9.51 µm |
| *Aspergillus fumigatus* | Ascomycetes | Blackish white margin | Regular | green | Fast | Powdery | Profusely branched, septate and hyaline | Long, erect hyphae each terminating in a bulbous hea,the vesicle. Size:80.87X6.79 µm | Round and formed inside the tip of the phialide. |
| *Cercospora ricinela* | Deuteromycetes | Grey green in the central part with white periphery | Central part uniformly distribute but the margin is irregular | Black | Slow | Flat | Direct hyaline hyphae | Clustered,dark conidiophores. | Dark.long.slender & multicelled |
| *Curvularia clavata* | Deuteromycetes | Dirty Brown | Irregular & floppy margin | Black | Very fast | Cottony | Septate & branching | Clustered | Septate oval& curved shaped conidia with paler end cells,size: 16.4X8.77µm. |
| *Fusarium solani* | Ascomycetes | White | Flat | White | | Cottony | Branching and hyaline | Macroconidia at the foot branche and the microconidia at the tip of the macroconidia. | Sickle shaped macroconidia with tapering end, single septed cell.Size:22.35X3 .79µm and oval shaped microconidia with 8.30X4.10µm size. |
| *Fusarium moniliform* | Ascomycetes | Light pink | Smooth to regular | Dark pink | Scattered/ clustered | Flat | Septate &Right angled branching | Erect and branched | Micro and macroconidia is found.microconidia in cluster with single celled and ovoid/oblong in shape. Macroconidia are hyaline, elongated, filiform and multisepted with pointed ends, measuring 14.3X5.02µm. |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Each macro-conidium gives rise to several germ tubes |
| *Leveillula taurica* | Ascomycetes | White | Irregular | Dirty white | fast | Powdery | Endophytic mycellium | Asci is formed | Conidia aboundant on the adaxial leaf surface,slendrical shaped with germinating tip Size:45X12µm |
| *Phytopthora parasitica* | Oomycetes | White with orange image | Cottony irregular | Light pink | Slow | Floppy | Coenocytic hyphae | Sporangia are semi papillate with long pedicel. | Lemon shaped,papillate sporangia with spiny like margin produce at the tip of sporangiophore.Size:18.5X18.7µm |
| *Penicillium sp.* | Ascomycetes | Olive green | Smooth | Dirty white | Fast | Powdery | Septate , branched,hyaline | Erect,unbranched,septate and biverticillate. | Conidia are round shaped arranged in a long chain,single celled,green coloured.size: |
| *Melampsora ricini* | Ascomycetes | Dirty brown | Floppy | Black with white margin | Fast | Fluppt | Septate | Produce sclerotia | Black coloured ascorspore unicellular,elliptic Size:2.3X3.2µm. |
| *Emericella nidulans* | Ascomycetes | Light green | Irregular | White | Fast | Powdery | Septate having multinuclear | Condiophore with sterigmata | Black clolored mold having vesicle with two raws of sterigmata |

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