Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5956

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (6):** 864-869 (2017)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Seedborne Nature and Transmission Studies of *Botrytis ricini* in Castor

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Received: 30.10.2017 | Revised: 28.11.2017 | Accepted: 2.12.2017

ABSTRACT

The seedborne nature of Botrytis ricini L. was tested using the component plating technique and transmission of B. ricini from castor (cv. DCS-9) seeds to seedlings was studied in laboratory as well as in glass house conditions. Component plating technique revealed that, B. ricini incidence was maximum in caruncle and seed coat in both naturally infected and artificially inoculated castor seeds. Whereas B. ricini incidence was totally absent in embryo in both naturally infected and artificially inoculated seeds. Studies on Transmission revealed that, seed rotting was maximum in naturally infected unsterilized seeds and germination percentage was higher in artificially inoculated sterilized seeds in paper towel. Seedling mortality and seedling infection were very low when compared to seed rotting. Whereas in sand and soil methods seedling infection was totally absent and seed rotting was maximum in naturally infected unsterilized seeds.

Keywords: *Component plating technique, transmission, seed rotting, seedling mortality.*

INTRODUCTION

Castor bean (*Ricinus communis* L.) is an important oilseed crop that belongs to the family Euphorbiaceae and has high oil content in seeds (46–55%). Its seed oil contains 90% of the unusual hydroxy-fatty acid ricinoleic acid which gives the highest viscosity and stability among vegetable oils which are used in manufacturing paints, lubricants, plastics, cosmetics , medical, and specialty chemical applications[11]. Further, castor bean is a potential biodiesel source and it can be cultivated in unfavorable environments, making it a promising crop in tropical developing countries. World's average total production of Castor seed figures around 12.5 lakh tons and is cultivated in more than 30 countries of the world. India is the world's largest producer of castor and its derivatives contributing to almost 65% share. The major exporters of castor oil are the leading producing countries of it namely India, China and Brazil from which only India has been successfully meeting the domestic and the world requirements. The states in the country that are major producers of castor are Gujarat, Andhra Pradesh, Rajasthan, Karnataka, Orissa, Tamil Nadu etc.

Cite this article: Siva, V.K, The Great, V.S. and Meena, K.V.S., Seedborne Nature and Transmission Studies of *Botrytis ricini* in Castor, *Int. J. Pure App. Biosci.* **5(6):** 864-869 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5956

The leading of them all is Gujarat, which contributes to 86% of the total castor seeds produced in the country. Andhra Pradesh and Rajasthan follow Gujarat in the production of castor seed. Andhra Pradesh relies on its districts namely Nalgonda, Mehboobnagar, Prakasam, Guntur and Ranga Reddy for the production of the state. The crop is attacked by about 150 pathogens that cause several diseases. Among these diseases, Seedling blight (*Phytophthora parasitica* Dastur), Alternaria leaf blight (*Alternaria ricini* Yoshii), Wilt (*Fusarium oxysporium f. sp. ricini* Schelecht), Root rot/dieback (*Macrophomina phaseolina* (Tassi) Goid.), Grey rot (*Botrtis ricini* Godfrey), Cercospora leaf spot (*Cercospora ricinella* Sacc. & Berl.), Bacterial leaf spot (*Xanthomonas campestris pv. ricini* (Elliot) Dow.) and Powdery mildew (*Leveillula taurica* (Lev.) Arn.) are important[15]. Of these grey rot caused by *Botrytis ricini* has been identified as the most destructive disease of castor as it directly infects the part of the plant, which is commercially valuable i.e., capsules and seeds from which the castor oil is obtained. Incidence of grey rot of castor was reported for the first time from India in 1985 and was identified to be caused by *Botrytis ricini*[1]. Grey rot incidence occurred at epidemic level with extensive damage of the crop in Andhra Pradesh during *kharif* 1987 which led to the decline in castor cultivation [6&8].Seed borne nature of castor grey mold was first reported by Godfrey (1923), which was further confirmed by Pietkiewicz and Kulkarni *et al.*,[5,7&12]. The fungus is found within the caruncle and even beneath the seed coat. The pathogen is known to survive in soil through sclerotia formed on diseased plant debris. The black sclerotia germinate by production of apothecia. Widespread epiphytotic appearance in short spells indicates definite modes of perpetuation. The pathogen is always present in the environment waiting for the development of congenial conditions.

Very little information is available regarding the seed borne nature and transmission of the pathogen from seed to seedling. In view of the non-availability of information on various aspects of the disease and considering the limitations associated in the control of spread of *B. ricini* in castor the present investigation was undertaken with the following objectives.

1. To detect *B. ricini* infection in different parts of castor seed.
2. To study the seed transmission of the fungus from infected castor seed to seedling

MATERIALS AND METHODS

Location of Seedborne Infection

The present investigation was carried out during 2010-11 in Department of Plant Pathology, College of Agriculture, Seed Research and Technology Centre and Directorate of Oilseeds Research, Rajendranagar, Hyderabad, Andhra Pradesh (India). One hundred castor seeds (cv. DCS-9) were tested using the component plating method as described by Maden *et al.*,[9]. The seeds were surface sterilized for 5 minutes in freshly prepared sodium hypochlorite solution (NaOCl) containing 1 % available chlorine and then washed thoroughly with sterile water. Each seed was separately soaked for 6-7 h in 1 ml of sterile water in plastic micro culture plates at room temperature (20-22˚C). Individual seed was dissected aseptically into seed coat, embryo, caruncle etc., and each component was plated separately on 3 layers of moistened blotters in plastic petri dishes. The dishes were incubated at 20˚ ± 1˚C under 12 h alternating cycles of NUV light and darkness. Each component was examined under a stereo microscope for the growth of B. ricini on the 7th and 10th day.

Transmission studies

Transmission of B. ricini from castor (cv. DCS-9) seeds to seedlings was studied in laboratory as well as in glass house conditions using both naturally infected as well as artificially inoculated seeds.

Laboratory studies

Paper towel method

For transmission studies under laboratory conditions, 400 seeds each of naturally infected and artificially inoculated seeds

(sterilized and unsterilized) were placed on paper towel, incubated at 25 ± 2° C and examined for symptom expression on seedlings after 10 days. Per cent germination, seed rot, seedling mortality and seedling infection was recorded.

Sand method

400 seeds each of naturally infected and artificially inoculated (sterilized and unsterilized) seeds were sown in sterilized sand in plastic trays and were monitored for germination and symptom expression on seedlings after 10 days. Per cent germination, seed rot, seedling mortality and seedling infection was recorded.

Glass house studies

Both naturally and artificially infected (sterilized and unsterilized) seeds were grown in sterilized substrate (soil + sand mixture) in earthen pots and were monitored for seedling growth and symptom expression at 15-30 days.

RESULTS AND DISCUSSION

Seed component plating technique revealed that infection of *Botrytis ricini* in the seeds of castor cv.DCS-9 is mainly located in the seed coat and in the caruncle region (**Table. 1**). The range of seed coat infection varied between 4.2 and 9.2% in naturally infected and artificially inoculated sterilized seeds, while that of caruncle varied between 11.2 and 7.5% in naturally infected and artificially inoculated sterilized seeds respectively.Naturally infected unsterilized castor seeds yielded higher percentage of seed coat as well as caruncle infection (7.5 and 15.0 per cent respectively) as compared to sterilized seeds (4.2 and 11.2 per cent). In the same way artificially inoculated unsterilized castor seeds yielded higher percentage of seed coat as well as caruncle infection (12.5 and 10.0 per cent) as compared to sterilized seeds (9.2 and 7.5 per cent) respectively.Among seed coat, endosperm and caruncle, *B. ricini* incidence was significantly higher in caruncle (15.0 and 11.2 per cent in unsterilized and sterilized seeds) when compared to seed coat (7.5 and 4.2 per cent) and endosperm (2.5 and 0.0%) in naturally infected seeds. In artificially inoculated seeds no significant difference was observed between caruncle infection (10.0 and 7.5 per cent in unsterilized and sterilized seeds) and seed coat infection (12.5 and 9.2 per cent).However, infection of the fungus was absent in embryo in both naturally infected and artificially inoculated seeds. A very low per cent endosperm infection (2.5 per cent) was seen in naturally infected unsterilized seeds. Whereas endosperm infection was completely absent in artificially inoculated seeds and naturally infected sterilized seeds. Similar findings were reported by Godfrey while working on blight of castor inflorescence[5]. He found *Botrytis* inside the caruncle and beneath the seed coat of seeds attacked before maturity.

Effect of *Botrytis ricini* infection on seed germination and pathogen transmission from seed to seedling

In paper towel

Seed transmission studies of the pathogen from infected castor (cv.DCS-9) seed to seedling under laboratory conditions in paper towel revealed that, naturally and artificially inoculated seeds with *B. ricini* showed seed rot, abnormal seedlings and in severely infected seeds seedling mortality thus resulting in reduced seed germination (**Table 2**). The germination in naturally infected castor seeds was 17.5 and 27.5 per cent in unsterilized and sterilized seeds respectively. In naturally infected seeds, 80 and 70 per cent seed rotting, 2.5 and 7.5 per cent diseased seedlings, 15 and 12.5 per cent seedling mortality were recorded in unsterilized and surface sterilized seeds respectively. The germination in artificially inoculated castor seeds was 72.5 and 85 per cent in unsterilized and surface sterilized seeds respectively. 22.5 and 12.5 per cent seed rotting, 5.0 and 5.0 per cent diseased seedlings and 7.5 and 5 per cent seedling mortality was observed in artificially inoculated seeds.Significant increase was observed in seed rotting in naturally infected seeds when compared to germination, seedling mortality and seedling infection. Due to severe infection of *B. ricini*, the seeds became hollow and

germination decreased significantly. In artificially inoculated seeds, germination percentage is significantly higher when compared to seed rotting because the pathogen could not penetrate into the seed due to hard seed coat and ultimately couldn't effect the germination.

In sand

Germination in naturally infected castor seeds was 8.0 and 15.5 per cent in unsterilized and surface sterilized seeds respectively. Similarly 83.5 and 78.0 per cent seed rotting, 5.0 and 2.5 per cent diseased seedlings, 11.0 and 5.0 per cent seedling mortality was observed in naturally infected sterilized and unsterilized seeds respectively. In artificially inoculated seeds germination percentage is 77.5 and 81.0 per cent in unsterilized and surface sterilized seeds. 14.5 and 11.5 per cent seed rotting, 5.0 and 2.5 per cent seedling mortality was observed. Seed transmission studies of the pathogen from infected castor (cv. DCS-9) seed to seedling under laboratory conditions in sand, revealed the similar symptoms that were observed in paper towel method (**Table 2**).Significant increase in seed rotting (83.5 and 78.0 per cent in unsterilized and surface sterilized seeds respectively) was observed when compared to seed germination (8.0 and 15.5 per cent) in naturally infected seeds. It may be due to complete rottening of the seed by *B. ricini* or may be due to incomplete filling of the seed, as the pathogen attacked the seed in early stages of growth. Whereas seed rotting was very low (14.5 and 11.5 per cent) in artificially inoculated seeds as the pathogen could not penetrate the hard seed coat, which prevented the pathogen to cause damage to the embryo and ultimately resulting in higher germination (77.5 and 81.0 per cent in unsterilized and surface sterilized seeds respectively).

Glass house studies In soil

Seed transmission studies of the pathogen from infected castor (cv. DCS-9) seed to seedling under glasshouse conditions in pots filled with soil, revealed the similar symptoms that were observed in sand (**Table 2**).In naturally infected seeds, germination was 9.5 and 17.5% in unsterilized and surface sterilized seeds respectively. Similarly 77.5 and 71.0% seed rotting, 5.0 and 2.5% diseased seedlings and 7.5 and 5.0% seedling mortality was recorded. In artificially inoculated seeds germination was 81.0 per cent in unsterilized seeds and 82.5 per cent in surface sterilized seeds whereas, 12.5 and 7.5 per cent seed rotting, 2.5 and 0.0 per cent seedling mortality was observed in unsterilized and sterilized seeds of castor cv. DCS-9.

TABLE 1: Incidence of *Botrytis ricini* in different components of infected seed of castor cv. DCS-9 using standard blotter method

Components of seed	Incidence of *Botrytis ricini* (%)			
	Naturally infected seeds		Artificially inoculated seeds	
	Unsterilized seeds*	Sterilized seeds*	Unsterilized seeds*	Sterilized seeds*
Seed coat	7.5 (15.8)	4.2 (11.8)	12.5 (20.7)	9.2 (17.6)
Embryo	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Endosperm	2.5 (9.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Caruncle	15.0 (22.7)	11.2 (19.5)	10.0 (18.5)	7.5 (15.8)
C.V. (%)	8.3	9.8	8.7	0.7
S.E.m ±	0.2	0.1	0.2	0.1
CD (P=0.05)	0.8	0.5	0.3	0.4

* Average of 400 seeds

Figures in the parenthesis are angular transformed values

TABLE 2 : Effect of *Botrytis ricini* infection on seed germination and pathogen transmission from seed to seedling in Paper towel, Sand and Soil

Test	Incidence of *Botrytis ricini* (%)											
	Paper towel method				Sand method				Glass house studies in soil			
	Naturally infected seeds		Artificially inoculated seeds		Naturally infected seeds		Artificially inoculated seeds		Naturally infected seeds		Artificially inoculated seeds	
	Unsterilized seeds*	Sterilized seeds*	Unsterilized seeds*	Sterilized seeds*	Unsterilized seeds*	Sterilized seeds*	Unsterilized seeds*	Sterilized seeds*	Unsterilized seeds*	Sterilized seeds*	Unsterilized seeds*	Sterilized seeds*
Germination %	17.5 (24.7)	27.5 (31.6)	72.5 (58.3)	85.0 (67.2)	8.0 (16.4)	15.5 (23.1)	77.5 (61.9)	81.0 (64.1)	9.5 (17.9)	17.5 (24.7)	81.0 (64.1)	82.5 (65.2)
Seed rot %	80.0 (63.4)	70.0 (56.7)	22.5 (27.9)	12.5 (20.7)	83.5 (66.0)	78.0 (62.0)	14.5 (21.9)	11.5 (19.3)	77.5 (61.6)	71.0 (57.4)	12.5 (20.7)	7.5 (15.8)
Seedling mortality %	15.0 (22.7)	12.5 (20.7)	7.5 (15.8)	5.0 (12.9)	11.0 (19.3)	5.0 (12.9)	5.0 (12.9)	2.5 (9.0)	7.5 (15.8)	5.0 (12.9)	2.5 (9.0)	0.0 (0.0)
Seedling infection %	2.5 (9.0)	7.5 (15.8)	5.0 (12.9)	5.0 (12.9)	5.0 (12.9)	2.5 (9.0)	0.0 (0.0)	0.0 (0.0)	5.0 (12.9)	2.5 (9.0)	0.0 (0.0)	0.0 (0.0)
C.V. (%)	0.2	0.2	0.1	0.2	3.4	3.5	3.1	0.1	2.2	3.4	0.1	2.1
S.E.m ±	3.0	2.9	2.6	2.7	0.4	0.4	0.3	0.4	0.2	0.4	0.2	0.2
CD (P=0.05)	9.4	9.1	8.0	8.3	1.4	1.3	1.2	1.2	0.8	1.2	0.8	0.7

* Average of 400 seeds

Figures in the parenthesis are angular transformed values

CONCLUSION

Experimental results revealed that, Seed caruncle is an outgrowth from integuments and is parenchymatous in nature. Being soft caruncle may thus provide a site for easy penetration for the pathogen. In immature capsules the developing seeds are attached to the wall by funiculus where vascular strands extend in seed and fuse with the seed coat. Seeds having such vascularisation possess particularly a favourable site for internal transmission of vascular pathogens[2].By comparing the seed to seedling transmission of *Botrytis ricini* in the three experiments i.e., paper towel, sand and soil methods it can be concluded that, in naturally infected seeds, seed rotting is significantly higher than germination since the seed may be ill-filled as the pathogen infected the seed during the early stages of seed formation or complete rotting of seed and making it hollow when the pathogen infected the seed at the time of maturation.Transmission of the fungus from seed to seedling is because the cotyledon tip remains attached to the seed coat upon germination which facilitates the inoculum of the fungus to invade the newly emerged seedling. Similar findings were reported by Trichlear *et al*., while working with transmission of *B. alli* (undifferentiated from *Botrytis aclada*) from seed to seedling[14]. He demonstrated microscopically that the fungus is able to invade the tip of the cotyledon from the seed coat. Similarly Brewster, J.L, stated that transmission of the fungus from seed to seedling is enhanced particularly because the cotyledon tip remains attached to the seed coat via a haustorium during germination and emergence, when the haustorium absorbs nutrients from the endodermis[3].However, Chilvers *et al*., Maude *et al*., and Stewart *et al*., reported that the level of *B.alli* infection detected in seedlings was significantly lower than that in the planted seed, suggesting that environmental conditions under which plants emerge may influence seed transmission of the pathogen from seed to seedling[4, 10&13].

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