J. Phytol. Res. **27** (1 & 2) : 57-65, 2014 **ISSN 0970-5767**

INCIDENCE AND TRANSMISSION OF *XANTHOMONAS AXONOPODIS* PV. *VESICATORIA* (DOIDGE) DYE IN BRINJAL (*SOLANUM MELONGENA* L.) SEEDS IN RAJASTHAN, INDIA.

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For the study of incidence and transmission of diseases of brinjal, a survey was conducted in various fields and market. Dry seed examination of 110 seed samples of brinjal (*Solanum melongena* L.) collected from 10 districts of Rajasthan revealed asymptomatic (07.75-97.50%), moderately discoloured (02.50-78.50%) and shriveled discoloured (04.25-95.50%) seed samples were studied. The discolouration varied from yellow to brown and black spots on seeds which yielded colonies of *Xanthomonas axonopodis* pv. *vesicatoria* on incubation. The standard cultural, biochemical and pathogenicity tests were carried out for identification of the bacterium.

Keywords: Bacterial transmission; Brinjal; Field survey; Incidence; Rajasthan.

Introduction

Brinjal (*Solanum melongena* L.) is the fourth most important vegetable grown after potato, onion and tomato in India[1,2]. It is an important crop of family solanaceae grown and consumed in the warm areas of Far East, being grown intensively in India, Bangladesh, Pakistan, China, Philippines, Egypt, France, Italy and United States. In India, the major brinjal producing states are West Bengal, Orissa, Bihar, Gujarat, Maharashtra, Karnataka, Uttar Pradesh and Andhra Pradesh with 13557.82 MT total production on 711.31 HA area. In Rajasthan, it is grown in Alwar, Kota, Jaipur, Sriganganagar and Bharatpur with accounts for about 23.22 MTs production with an area of 5.54 ha[3,4]. The pathogen *Xanthomonas axonopodis* pv. *vesicatora* (Doidge) Dye (syn: *Xanthomonas campestris* pv *vesicatoria*) (XAV) is a gram-negative, rod-shaped bacterium that attack and produce symptoms aerial part of plant as leaf spots and fruit spots.

It has ayurvedic as well as medicinal properties, good for diabetic patients and recommended as an excellent remedy for those suffering from liver complaints. The fruit does not contain endogenous toxins or a significant level of anti-nutritional factors causing any disease in human, animals or plants[2]. In Brazil, it is consumed extensively and believed that infusion of a powdered preparation of the fruit may reduce serum

cholesterol. The results suggest that infusion of brinjal has modest and transitory effect which was not different from the obtained with standard and orientation for dyslypidemia patients (diet and physical activities). The aqueous extract of peduncles used frequently as mouth and benefited effect against periodontal disease. Ethano-botanically, the plant used for cure of several diseases by using different parts of plants such as fruit stalk (fistula and piles), mature fruit (stomach pain) and leaf (burns)[8,9].

In the present study, incidence and transmission of pathogen in seed samples of brinjal from Rajasthan state have been studied and bacterial species identified by available record and detailed description described by various scientists[4-6].

Material and Methods

Identification and incidence of the pathogen in seed samples: One hundred ten seed samples of brinjal collected from 10 district of Rajasthan were studied by dry seed examination, incubation on moistened blotters[10] and Tween-80 agar plate method[11,12] to find the incidence of *Xanthomonas axonopodis* pv. *vesicatoria* in seed samples. After 72 hrs of incubation at 30°C[13,14] typical bacterial colonies from seeds raised on Tween-80 agar plate were transferred to Tween-80 agar medium to obtain pure cultures. These pure colonies were subjected to various tests namely gram's staining, KOH solubility test, levan formation, oxidase test[15,16], potato soft rot test, nitrate reductase test[17], arginine dihydrolysis, gelatin hydrolysis test, hypersensitivity test in tobacco and pathogenicity tests[11] for the identification of the bacterial species. For all the tests 24-48 hrs old cultures[11] and bacterial suspensions[18] were used. The bacterial isolates identified by various methods as described above were subjected to pathogenicity tests[4,7] on the host plant and other plant species.

Disease transmission: Two naturally infected seed samples of brinjal (Lab. ac. nos. SM-O15 and SM-O22) carrying 80% and 89% infection of *Xanthomonas axonopodis* pv. *vesicatoria* were selected for transmission studies. 100 seeds per category per sample were sown on moist blotters (10 seeds/plate) and 1% water agar medium in test tubes (1 seed/test tube) incubated at 25±2°C for 12/12 h alternating cycles of light and darkness up to 7 days and 14 days per category per sample were sown in pots (5 seeds/pot) and data on percent seed germination, ungerminated seeds associated with the pathogen (bacterial colonies), seedling symptoms and mortality were recorded. Isolation of the pathogens was carried out from the infected plant parts at different stages of plant growth.

Pathogenicity test: Artificial inoculation of the bacterial isolates was carried out by techniques of incubation of smoothed seeds and stab inoculation of seedlings and other parts of the plants.

Result and Discussion

Identification of the pathogen and its incidence: Total 110 seed samples of brinjal collected from different districts of Rajasthan revealed asymptomatic (07.75-97.50%), moderately discoloured (02.5-78.50%) and shriveled discoloured (04.25-95.50%) seeds. The discolourations varied from light brown to black and water-soaked translucent shining areas on seed surface during dry seed examinations and such symptomatic seeds on incubation yielded the growth of *Xanthomonas axonopodis* pv. *vesicatoria* (XCV). Similar symptoms on seeds were observed previously caused by XCV in rape and mustard[19], in chilli[19-21], by *Xanthomonas campestris* pv. *cajani* in

Table 1. Incidence of *Xanthomonas campestris* pv. *vesicatoria* in seeds of brinjal grown in Rajasthan state, India

S. No.	Name of Districts	Total no. of samples collected
1.	Jaipur	67
2.	Tonk	4
3.	Jhunjhunu	6
4.	Kota	5
5.	Ajmer	3
6.	Sikar	6
7.	Jalor	6
8.	Nagaur	3
9.	Bikaner	8
10.	Jodhpur	2
	Total	**110**

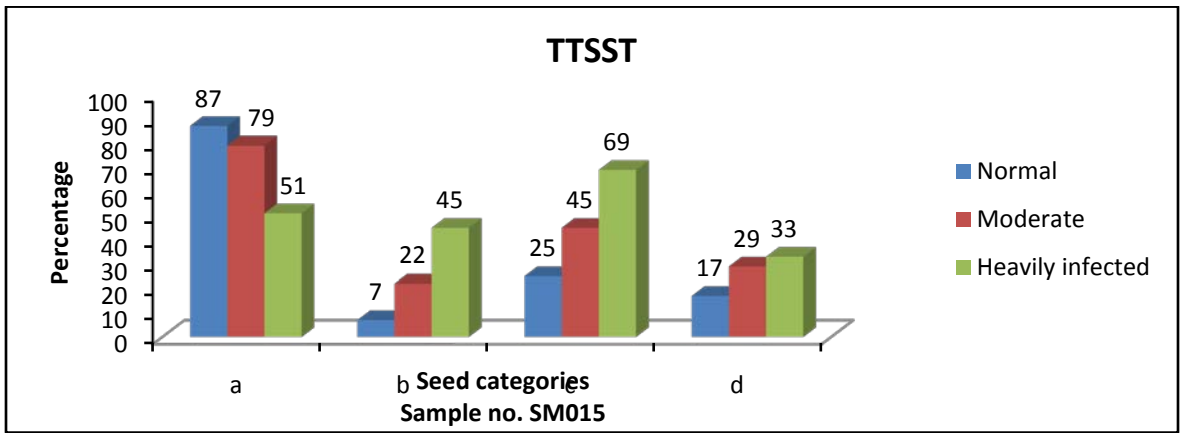
pigeon pea[23,24] and by *X. anonopodis* pv. *cyamopsidis* in cluster bean[25,26].

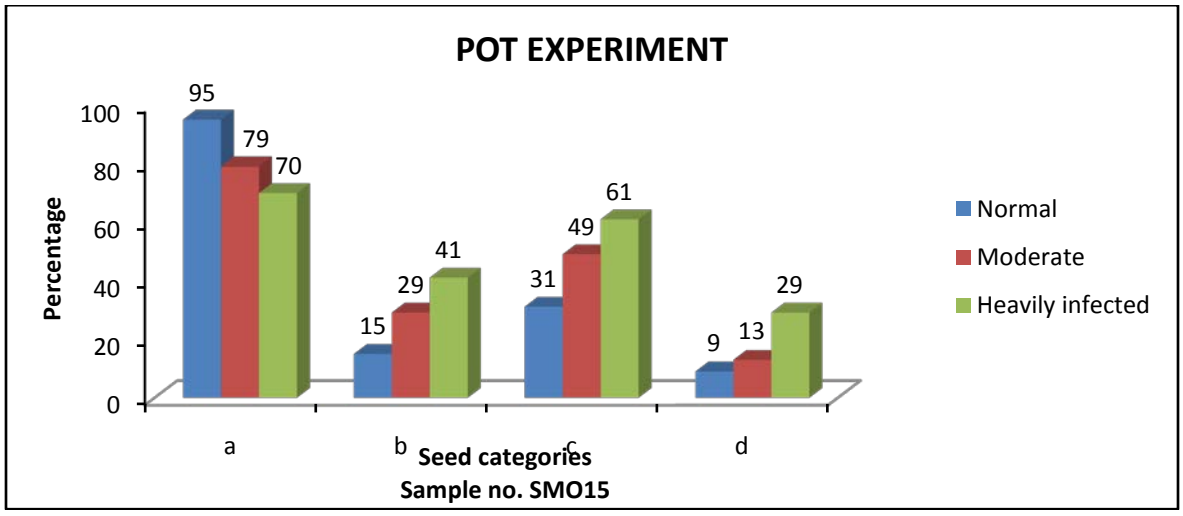
The bacterial colonies isolated from various seed samples were produced as circular, raised, yellow, mucoid colonies on Tween-80 agar medium and identified to be *Xanthomonas axonopodis* pv. *vesicatoria*. The isolates were gram's negative, KOH solubility test positive, levan negative, lipase activity positive, oxidase negative, starch hydrolyzing, gelatin hydrolyzing, arginine variable, did not reduce nitrate and no rotting of potato tissue occurred. The pathogen induced positive hypersensitivity reaction on tobacco leaves after infiltration and turgidity of leaves was lost within 6-10hrs followed by local necrosis and desiccation of affected leaf tissues after 36 hrs.

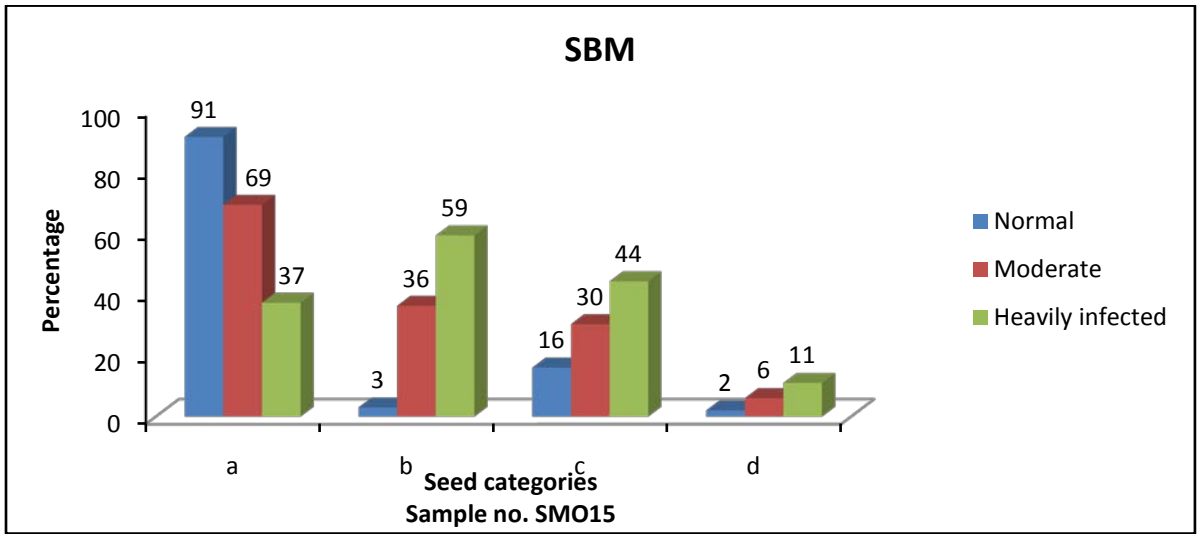
Disease transmission

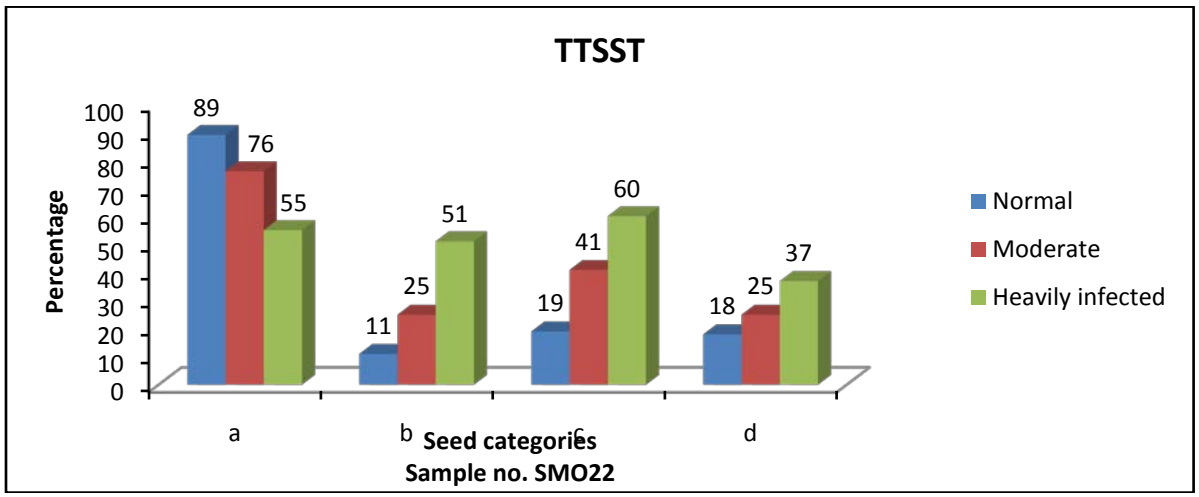
Petriplate method: The radical emergency in seed started after 48 hrs and germination completed within 5 days of incubation. It was 91, 69 and 37% in sample ac. no. SM-O15 and 98, 64 and 39% is ac. no. SM-O22 in asymptomatic, moderately discoloured and shrivelled discoloured seeds respectively. The hypocotyle showed rotting of apical shoot, the roots showed puffing/bulbous growth and burning at the end which showed rotting ultimately (Fig. 1-f). The shrivelled discoloured or infected seeds failed to germinate and yielded heavy growth of pathogen on and around on the seed on semi-selective medium. Such seeds were 3, 36 and 59% and 6, 39 and 60% in the three categories in both the samples respectively.

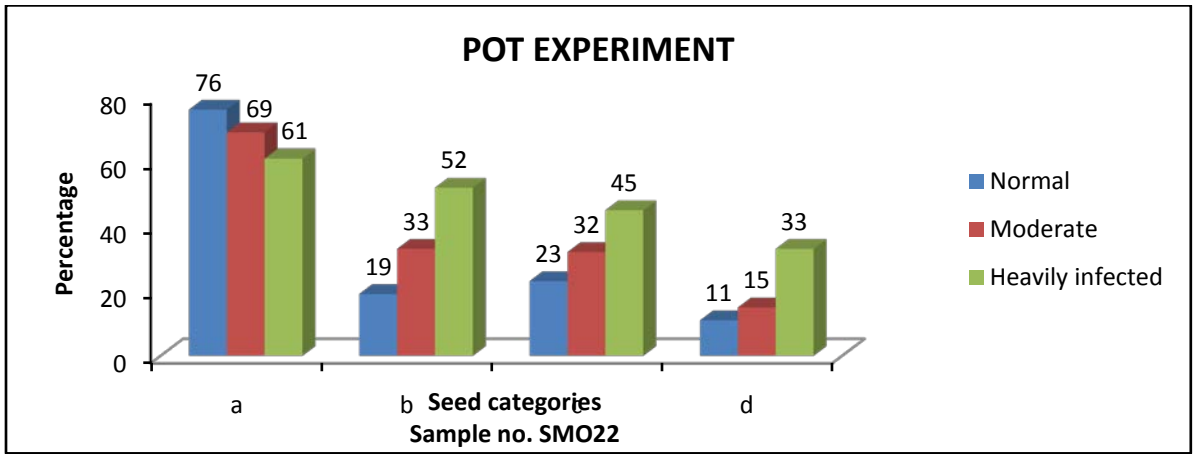
Water agar test tube seedling symptom test: On water agar, the seed germination was 87, 79 and 51% (ac. no. SM-O15) in asymptomatic, moderately and heavily discoloured seeds respectively on the 15th day of inoculation. The percentage of ungerminated seeds was 11, 25 and 51% (ac. no. SM-O15) and 11, 41 and 65% (ac. no.

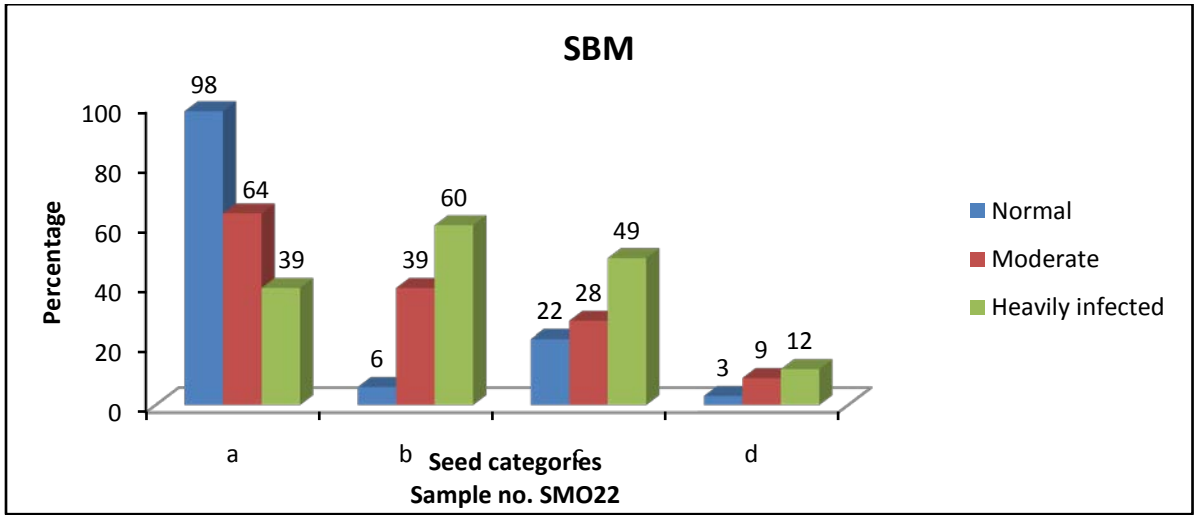

TTSST
Percentage
100
90
80
70
60
50
40
30
20
10
0
87
79
51
7
22
45
25
45
69
17
29
33
a
b
c
d
Seed categories
Sample no. SM015
Normal
Moderate
Heavily infected


POT EXPERIMENT
Percentage
100
80
60
40
20
0
95
79
70
15
29
41
31
49
61
9
13
29
a
b
c
d
Seed categories
Sample no. SMO15
Normal
Moderate
Heavily infected


SBM
Percentage
100
80
60
40
20
0
91
69
37
3
36
59
16
30
44
2
6
11
a
b
c
d
Seed categories
Sample no. SMO15
Normal
Moderate
Heavily infected







a = Seed germination; b = Ungerminated seeds with pathogen; c = Symptomatic seedlings; d = Seedling mortality

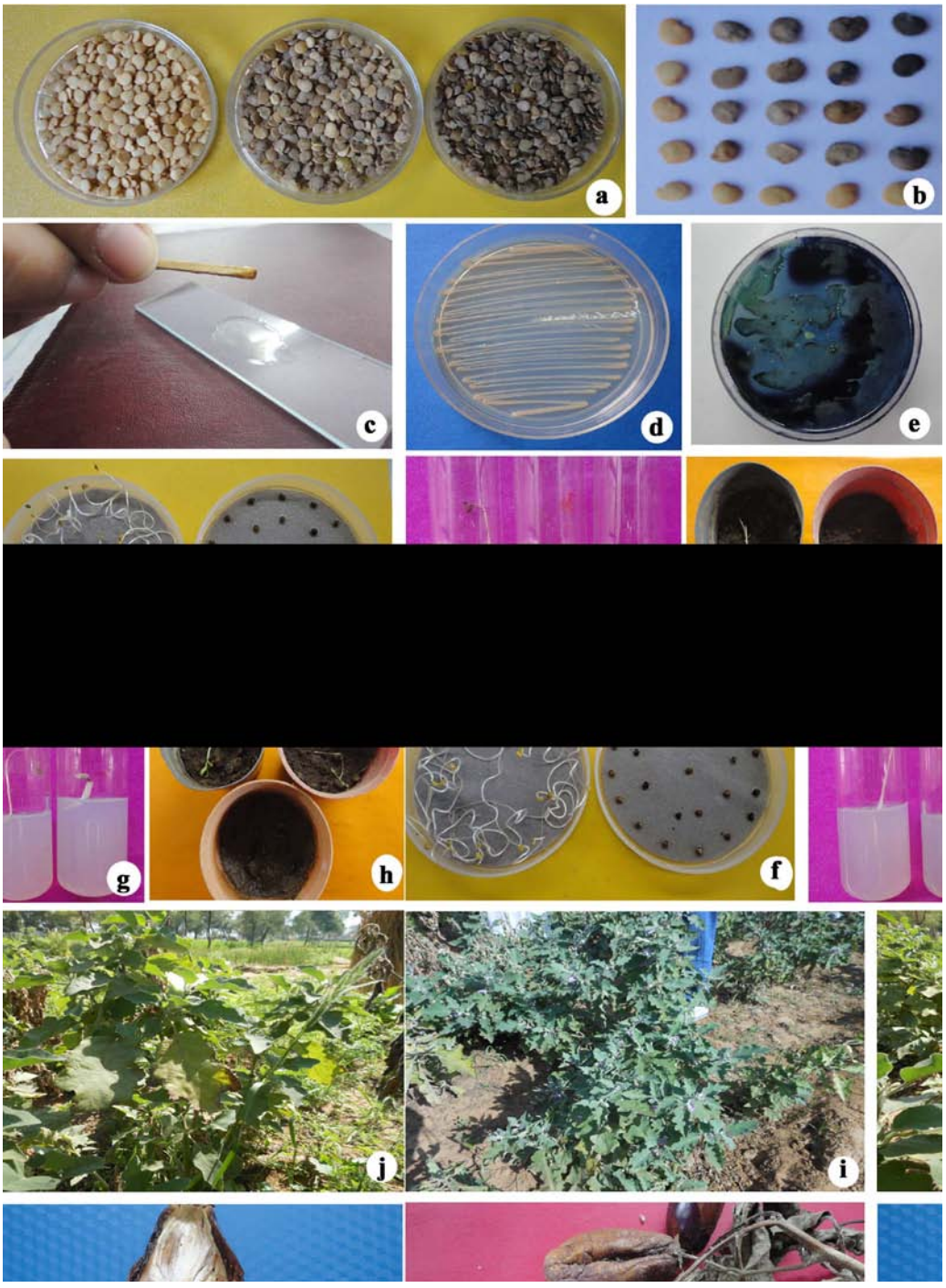


Fig. 1. Infection of *Xanthomonas campestris* pv. *vesicatoria* in brinjal (a,b) Seeds categorization into asymptomatic (upper left), moderately discoloured (middle one) and shrivelled discoloured seeds (upper right) showing degree of discolourations, (c) KOH solubility test positive, (d) Characteristic yellow colonies on NA agar medium, (e) Bacterial isolate showing hydrolysis of starch on modified MSX medium, (f) Seeds on blotter plate, (g) Water agar test tube seedling symptom test, (h) Pot experiment, (i,j) Field survey, (k,l) Symptoms on stem, leaf and fruit.

SM-O22) in the three categories respectively. The symptomatic seedlings showed burning and rotting at hypocotyls region such as observed in petriplate method. The mortality of seedlings on 15th day was the maximum in shriveled or heavily discoloured seeds to be 33 and 37% as compared to moderately discoloured seeds (29 and 25%) in ac. no. SM-O15 and SM-O22 respectively (Fig. 1g). Similar results were observation in sunflower[27], brinjal[22], tomato and chilli[20,28], soybean[29], crucifer seeds[30] and pigeon pea[31].

Pot experiment: The seed germination started on 10 day of showing in the pot experiment and continued up to 45 days in symptomatic was 95, 79 and 70% in ac. no. SM-O15 and 76, 69 and 61% in ac. no. SM-O22 in the three seed samples were appeared in 5-8 days after seed germination, which included small circular irregular water-soaked areas showing definite spots on lower surface of leaf. The survival of infected plants was 58, 35 and 31% and 89, 67 and 34% in both the samples in the said three categories, respectively (Fig. 1-h).

Pathogenicity tests: On smothering (rolling of healthy seeds in pure culture of bacteria) of healthy seeds of brinjal with the pure culture of the pathogen in petri plate method and water agar test tube seedling symptoms test, the seedlings raised, showed browning and puffing at radical followed by rotting and ultimately mortality. The mortality was 67.24 and 53.35% in petri plate method while 35.27 and 47.07% in water agar test tube seedling symptom test in both samples, respectively.

Acknowledgement

Authors are grateful to Prof. Ashok Sharma, hon'ble vice-chancellor, VMOU, Kota, Prof. Kailash Agrawal Head, Department of Botany, University of Rajasthan, Jaipur, faculty members of P.G. Department of Botany for valuable support and academic guidance. Thanks to Dr. Meena, Sh. Kailash Chaudhrary and Sh Mahesh Kumar for the valuable technical support in preparing this research article.

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