Detection of seed borne mycoflora seeds of two medicinal plants *Diplocyclos palmatus* (L.) Jeffrey and *Psoralea corylifolia* L.

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Abstract: *Diplocyclos palmatus* (L.) Jeffrey and *Psoralea corylifolia* L. are the two important medicinally plants. *D. palmatus* belongs to family Cucurbitaceae. These seeds are used to treat many diseases but some fungi spoil these seeds causing a great loss to human beings. There are no previous reports of mycoflora on seeds of *D. palmatus* and *P. corylifolia*. Among the fungi genera isolated from the seeds of *D. palmatus* were *Aspergillus Sp*., *Fusarium, Mucor spp., Pythium proliferatum* and *Rhizopus oryzae,* and on seeds of *P. corylifolia*, *Aspergillus Spp*., *Fusarium, Mucor spp., Pseudallescheria boydii* and *Rhizopus oryzae*. Seeds show that fungus *Aspergillus Sp*. and *Rhizopus oryzae* had very high percentage of infection and *Fusarium, Mucor spp., Pythium proliferatum* and *Pseudallescheria boydii* had very low percentage of infection.
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1. Introduction

Diplocyclos palmatus (L.) Jeffrey and *Psoralea corylifolia* L. are the two important medicinally plants. *Diplocyclos palmatus* (L.) Jeffrey *syn Bryonia palmate. Zehneria erthrocarpa, Bryonopsis laciniosa* is an annual herb. Its belongs to family Cucurbitaceae. It is commonly known as Shivalingi, Gargumaru, Ishwaraligi and Lillipop plant. Plant Shivlingi shows antiasthmatic, analgesic and anticovulsant activities. **(Reddy *et al.*, 2010).** *Diplocyclos palmatus* is used to treate asthma, bronchitis, cholera, colic, fever, megalospleny, paralysis, pluritic and pumonic disorders, snake-bite, tuberculosis, rheumatoid arthritis etc. **(Caroline and Mallaiah, 2011; Gupta *et al.*, 2003)**. Seeds of this plant are also used in female infertility. Seeds is reported as potent medication in healing several ailments **(Ehsan *et al.*, 2009)**. *Psoralea corylifolia.* L, an annual herbaceous plant and is about 1m. in height. It belongs to family Fabaceae (Papillionaceae). Common names of *Psoralea corylifolia* are babchi, bawchi, hakuchi, kantaka, karpokarishi, krishnaphala and vakuchi. Seeds of *P. corylifolia* have very much medicinal value. Seed oil is extremely beneficial and used externally in numerous skin ailments. Seeds extract is used as a constituent of Safi and Purim **(Uikey *et al.*, 2010)**. In chronic skin disease, a mixture of bakuchi and kranja oil is commonly used with Vaseline, Scabies, psoriasis, ring worm and tinea versicular are treated successfully with bakuchi. Seeds of *P. corylifolia* also use in leucoderma Seeds of *P. corylifolia* also have some economic value like making perfumes, preservatives for pickles in Japan **(Nadkarni, 1976; Qiao *et al.*, 2007)**. All seeds lots are known to carry a wide range of microorganism on their surface which under favourable conditions germinate and cause a considerable damage to seed. Theses fungi are parasites as well as saprophytes on the seeds. These are the first report of occurrence of fungal group of seeds of *D. palmatus* and *P. corylifolia.*

2. Materials and Methods

Seed samples collected from different seed stores were used for the isolation and detection of seed mycoflora of *Diplocyclos palmatus* (L.) Jeffrey and *Psoralea corylifolia* L. by agar plate method, blotter method with some modification as per recommended by ISTA (Anon 1966) and deep freezing method (Limonard, 1968) were used for the isolation of fungi.

Agar plate method- 100-100 Seeds of *Diplocyclos palmatus* (L.) Jeffrey and *Psoralea corylifolia* L. sterilized with1% sodium hypochlorite solution for 1-2 minute followed by washing with sterilized water. Such seeds were placed in the petridishes containing 20 ml PDA (10 seeds/petridish). The plates were incubated for 7 days at 25 $\pm 1^0$C under 12 hrs alternating cycle of near ulteraviolet light and darkness. Then the petridish was examined with the help of stereobinocular microscope.

Blotter method- Seeds of *Diplocyclos palmatus* (L.) Jeffrey and *Psoralea corylifolia* L. sterilized with1% sodium hypochlorite solution for 30 seconds followed by washing with sterilized water 3-4 times.

Such seeds were placed in the petridishes containing 3 layers of moistend blotters (10 seeds/petridish). The plates were incubated for 7 days at 25 $\pm 1^0$C under 12 hrs alternating cycle of near ulteraviolet light and darkness. Then the petridishes was examined with the help of stereobinocular microscope.

Deep freezing method- Seeds were placed at the rate of 10 seeds per plate on moistened blotter. Petridishes were incubated at 25 $\pm 1^0$C for 24 hour under 12 hrs alternating cycle of near ulteraviolet light and darkness, for next 24 hour and then plates were incubate at -20^{0}C in dark and were kept back under original condition for the next 6 days. After eight days of incubation seeds were examined with the help of stereo binocular microscope.

The fungi were identified after reference to Raper and Thom, 1949; Booth, 1971; Ellis, 1971.

3. Result and Discussion

Table 1 envisages that in all total 10 fungal species were isolated from seeds of *Diplocyclos palmatus.* 09 fungal species were isolated from agar plate method on the seeds viz. *Aspergillus fumigate* showed 10 percent, *Aspergillus flavus* showed 05 percent *A. niger* showed 19 percent, *A. terreu* showed 01 percent, *A. ustus* showed 01 percent, *Fusarium spp.* showed 05 percent, *Pythium proliferatum* showed 02 percent, *Mucor spp.* showed 10 percent and *Rhizopus oryzae* showed 42 percent.07 species were found only from blotter paper method viz *A. fumigates* showed 05 percent, *A. niger* showed 36 percent, *A. terreus* showed 05 percent, *A.ustus* showed 10 percent, *Fusarium spp.* showed 10 percent, *Mucor spp.* showed 25 percent and *Rhizopus oryzae* showed 13 percent. 06 species isolated from deep freezing method viz. *A. fumigates* showed 06 percent, *A. niger* showed 09 percent, *A. terreus* showed 14 percent, *Fusarium spp.* showed 14 percent, *Mucor spp.* showed 30 percent and *Rhizopus oryzae* showed 13 percent. *A. fumigates, A. niger, A.terreus, Fusarium spp., Mucor spp. and Rhizopus oryzae* isolated from all methods**.** All methods showed that *Rhizopus oryzae, A. niger* and *Mucor spp.* had highest percentage 42%, 36% and 30% respectively (Fig. 1) of infection on seeds and *A.terreus, A.ustus A.terreus, A. fumigatus* and *A. fumigatus* had lowest percentage 1%, 1%, 5%, 5% and 6% respectively (Fig. 1) of infection in Shivlingi seeds. Table 1 also envisages that in all total 10 fungal species were isolated from seeds of *Psoralea corylifolia* 08 fungal species were isolated from agar plate method on the seeds viz. *Aspergillus fumigate* showed 05 percent, *Aspergillus flavus* showed 05 percent, *A. niger* showed 58 percent, *A. terreu* showed 02 percent, *A. ustus* showed 05 percent, *Pseudallescheria boydii* showed 03 percent, *Mucor spp.* showed 04 percent and *Rhizopus oryzae* showed 07 percent.05 species were found only from blotter paper method viz *Aspergillus flavus* showed 09 percent, *A. fumigates* showed 09 percent, *A. niger* showed 41 percent, *Mucor spp.* showed 13 percent and *Rhizopus oryzae* showed 10 percent. 05 species isolated from deep freezing method viz. *Aspergillus flavus* showed 09 percent *A. fumigates* showed 28 percent, *A. niger* showed 14 percent, *Mucor spp.* showed 15 percent and *Rhizopus oryzae* showed 14 percent. *Aspergillus flavus, A. fumigates, A. niger, A.terreus, Mucor spp. and Rhizopus oryzae* isolated from all methods**.** All methods showed that *A. niger, A. niger* and *A. fumigatus* had highest percentage 58%, 41% and 28% respectively (Fig. 2) of infection and *A.terreus, A.flavus, A. fumigatus* and *A.flavus* had lowest percentage 2%, 9%, 9% and 9% respectively (fig. 2) of infection in babchi seeds.

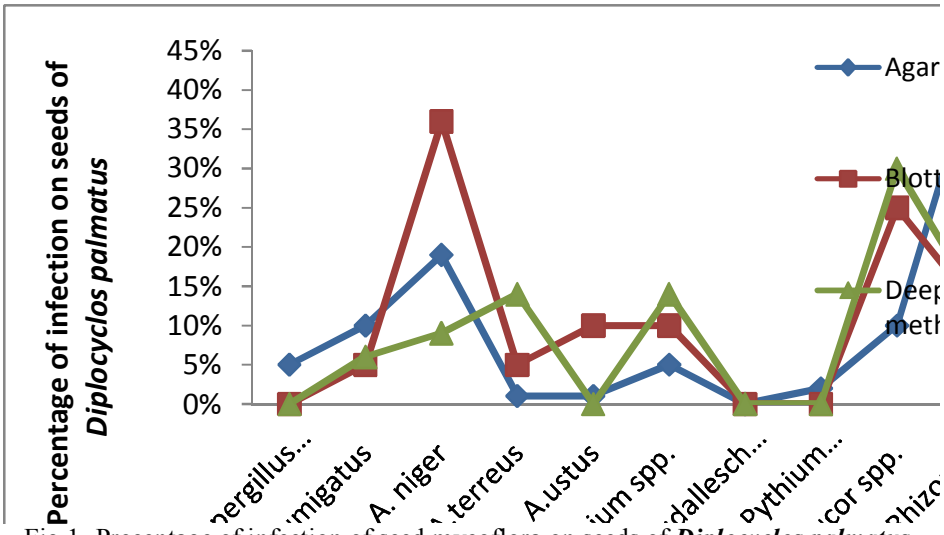


Fig.1- Precentage of infection of seed mycoflora on seeds of ***Diplocyclos palmatus***

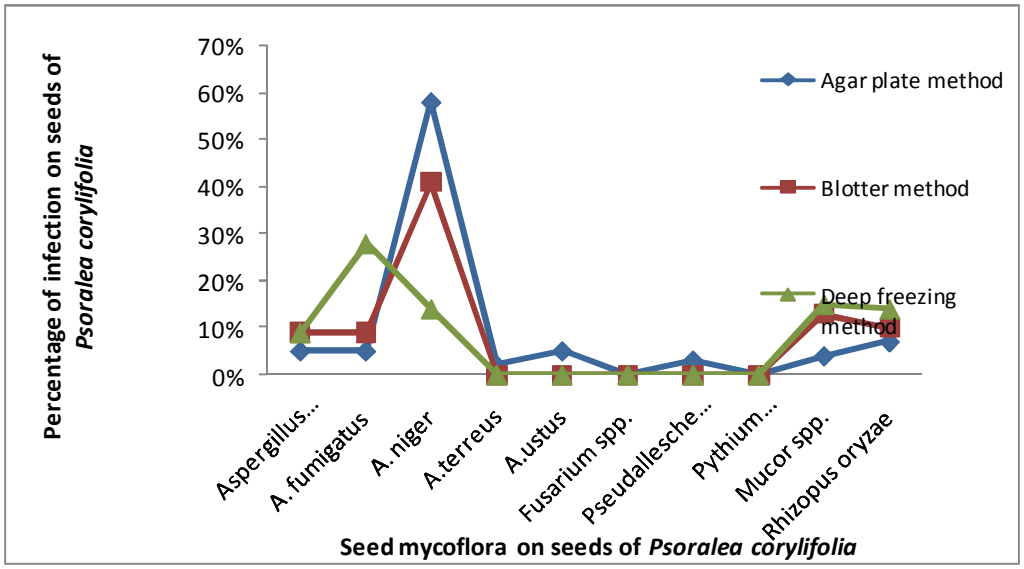
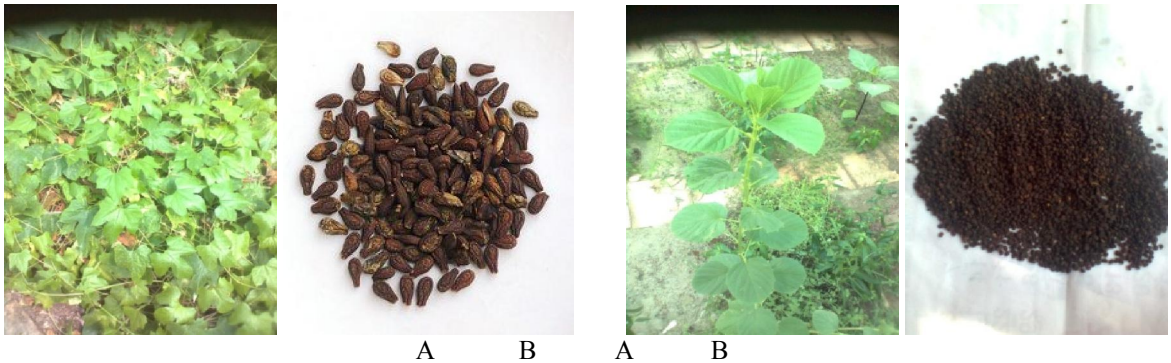


Fig.1- Precentage of infection of seed mycoflora on seeds of ***Psoralea corylifolia***

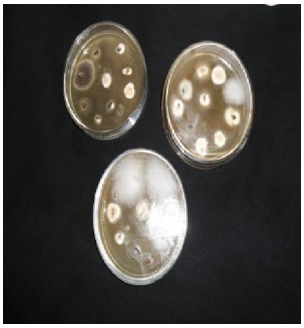
Table -1 Shows seed mycoflora of *Diplocyclos palmatus* and *Psoralea corylifolia*

S.no.	Seed mycoflora	Percentage of infection in seeds					
		Diplocyclos palmatus			*Psoralea corylifolia*		
		A	**B**	**DF**	A	B	DF
1.	*Aspergillus flavus*	05%	Nil	Nil	05%	09%	09%
2.	*A. fumigatus*	10%	05%	06%	05%	09%	28%
3.	*A. niger*	19%	36%	09%	58%	41%	14%
4.	*A.terreus*	01%	05%	14%	02%	Nil	Nil
5.	*A.ustus*	01%	10%	Nil	05%	Nil	Nil
6.	*Fusarium spp.*	05%	10%	14%	Nil	Nil	Nil
7.	*Pseudallescheria boydii*	Nil	Nil	Nil	03%	Nil	Nil
8.	*Pythium proliferatum*	02%	Nil	Nil	Nil	Nil	Nil
9.	*Mucor spp.*	10%	25%	30%	04%	13%	15%
10.	*Rhizopus oryzae*	42%	13%	13%	07%	10%	14%

A- Agar plate method B- Blotter paper method DF- Deep freezing method



A B A B



C D

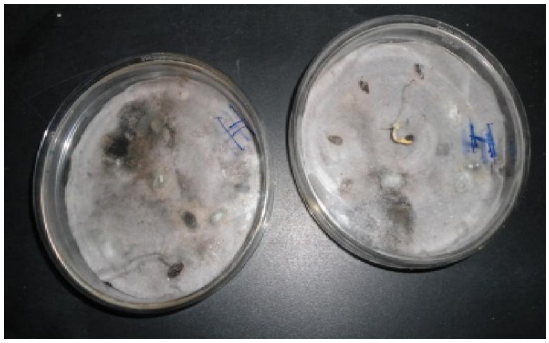
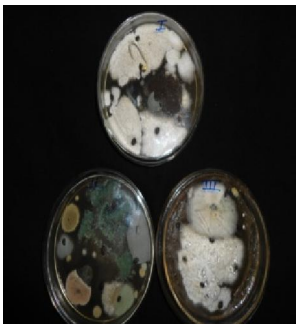

E

PLATE-1

A-Shows whole plant of *Diplocyclos palmatus*
B- Shows seeds of *Diplocyclos palmatus*
C-Shows seeds of *D. palmatus* incubate by Agar plat method
D- Shows seeds of *D. palmatus* incubate by Blotter method
E- Shows seeds of *D. palmatus* incubate by Deep freezing method



C D


E

PLATE-2

A- Shows whole plant of *Psoralea corylifolia*
B-Shows seeds of *Psoralea corylifolia*
C- Shows seeds of *P. corylifolia* incubate by Agar plat method
D- Shows seeds of *P. corylifolia* incubate by Blotter method
E- Shows seeds of *P. corylifolia* incubate by Deep freezing method

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Reference

1. Abdullah, S.K. and Kadhum, S.A. (1987). Seed mycoflora of *Sorghum bicolor* in Iraq. *Art Gulf J. Sci. Res*., 5(3): 401-410.
2. Asalmol, M.N., Kale, V.P. and Ingle, S.T. (2001). Seed borne fungi of chilli, incidence and effect on seed germination. *Seed Res*. 29(1): 76-79.
3. Bhale, M.S., Khare, D., Raut, N.D. and Singh, D. (2001). Seed borne diseases objectionable in seed production and their management, *Scientific Publishers*, p.186.
4. Caroline, V.J.E. and Mallaiah, B. (2011) High frequency *in vitro* rhizogenesis in *Bryonopsis laciniosa* (L.) Naud. A highly valuable medicinal Cucurbit. *Inter. J. of Pherma and Bio Science.* 2(1): B216-B223.
5. Dawar, S. and Ghaffar, A. (1991). Detection of seed borne mycoflora of sunflower. *Pak. J. Bot*., 23: 173-178.
6. Ehsan, B.R., Vital, A. and Bipinraj, N.K. (2009). Antimicrobial activity of the ethanolic extract of *Bryonopsis laciniosa* leaf, stem, fruit and seed. *African Journal of Biotechnology.* 8(15): 3565-3567.
7. Gaur, R. B. and Ahmed, S.R. (1984). Seed mycoflora of four varieties of Raya (*Brassica juncea). Indian J. Mycol Plant Pathol*. 12(2): 225-226.

8. Gupta, M., Sivakumar, T., Mazumdar, U.K., Vamsi, M.L., Karki, S.S., Sambathkumar, R. and Manikandan, L. (2003). Evaluation of anti-inflammatory activity of chloroform extract of *Bryonia laciniosa* in experimental animal models. *Biol. Pharm. Bull*. 26(9): 1342 1344.
9. I.S.T.A. (1985). International Rules for Seed Testing Chief Editor S.R. Draper. *Seed Test Asso*. Zurich, Switzerland. 1-520.
10. Khan, S.A.J., Khanzada, A.K., Sultana, N. and Aslam, M. (1988). Evaluation of seed health testing techniques for the assessment of seed borne mycoflora of rice. *Pak. J. Agric. Res*., 9: 502-505.
11. Nadkarni, K.M. (1976). Mumbai: Popular Prakashan Pvt. Ltd. *Indian Materia Medica*.1: 1019-22.
12. Nasir, N. (2003). Detecting seed borne fungi of soybean by different incubation methods. *Plant Pathol. J*. 2: 114-118.
13. Neergaard P. (1977). Seed Pathology. The Macmillian Press Ltd. London, UK, 3: 519-839.
14. Neergaard P. (1981). Seed transmitted crop diseases. *Science and Scientist.* 327-334.
15. Qiao, C.F., Han, Q.B., Song, J.Z., Mo, S.F., Kong, L.D. and Kung, H.F. (2007). Chemical fingerprint and quantitative analysis of fructus psoraleae by high-performance liquid chromatography. *J Sep Sci.* 30: 813-8.
16. Reddy, J., Gnanasekaran, D., Vijay, D. and Ranganathan T.V. (2010). *In vitro* studies on anti asthmatic, analgesic and anti convulsant activities of the medicinal plant *Bryonia laciniosa.Linn. International Journal of Drug Discovery.* 2(2): 01-10.
17. Uikey1, S.K., Yadav, A.S., Sharma, A. K., Rai, A.K., Raghuwanshi, D.K. and Badkhane, Y. (2010). The Botany, Chemistry, Pharmacological and Therapeutic Application of *Psoralea corylifolia* L. *International Journal of Phytomedicine.* 2: 100-107.

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