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In-vivo Testing of Plant Extracts against Seed borne Pathogens

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Abstract

Seed as a biological entity and a basic agricultural input, is the end product of plant growth by which plant life is regenerated. Plant disease causing pathogens are mostly seed borne and seed transmitted. Seed borne pathogens cause diseases of seed, seedling, and adult plants at various growth stages. Seeds are treated by various means to get rid of such pathogens, physical, chemical, and biological methods are available for treatment. Biological method need preference since plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides. Present paper deals with effectiveness of the plant products against the pathogens. The preliminary screening of a large number of plants is done by in vitro method and the promising ones are tested in vivo.

Keywords: Biological entity, seed borne disease*, in vitro, in vivo, t*ransmission, management.

Introduction

The most vital input in crop production programme is seed, it should be of high quality and pathogen free. Pathogen free sound seeds are preffered for sowing to have desired germination, emergence, health seedlings and plant population. Fungi form the largest group among such microorganisms causing seed damage, seed rot diseases at later stages of crop growth til maturity Seed borne fungi may be present in form of hyphae, conidia, oospores, chlamydospores, sclerotia, microspores, hyalospores and phaeospores. Seeds provide natural substrate for the growth of associated fungi, they get associated with seed externally on the seed surface, seed coat and internally with the endosperm, cotyledons, plumule ,radical, embryo. Some are on the seed surface as contaminant this influences the seed to plant transmission of the pathogen. Seed borne pathogens result in heavy losses in crop yield and seed quality. Management of seed borne pathogens is the need of hour. Biological control of plant pathogens is preferred over the hazardous chemical based products. Botanicals like leaf, root, stem, rhizomes, bulbs and other plant parts are used as extracts to control seed borne pathogens by seed treatment. There is an urgent need to find out effective, alternative methods of diseases control, which are less harmful to human beings and environment. In view of these the present investigation was undertaken to screen some plant part extracts against seed borne pathogenic fungi and the data has been presented in this paper.

A special feature of higher angiospermic plants is their capacity to synthesise aromatic substances, most of which are phenols or their derivatives. Most are secondary metabolites of which atleast 12,000 have been isolated. In many cases, these substances serve as plant defence mechanisms against microorganisms, insects and herbivores. Some phenols such as terpenoids give plant their odor, other i.e. quinines and tannins are responsible for plant pigmentation. Many compounds are responsible for plant flavor like terpenoids, capsaicin from Chilli etc.

Plants synthesize a bewildering variety of phytochemicals like:

Alkaloids - They contain a ring with nitrogen, many alkaloids have dramatic effects on the central nervous system. Caffeine is an alkaloid that as a mild lift but alkaloids in Datura cause severe intoxication and even death.

Phenolics - They contain phenol rings. The anthocyanins that give grapes their purple colour, the isoflavones, the phytoestrogens from soy and the tannins that give tea its astringency are phenolics.

Terpenoids - They are built up from terpene building blocks. Each terpene consists of two paired isoprenes. The names monoterpenes, sesquiterpenes, diterpenes , and triterpenes are based on the number of isoprene units. The fragrance of rose and lavender is due to monoterpenes. Carotenoids produce the reds, yellows and oranges of pumpkin, corn and tomatoes.

Glycosoides – It consists of a glucose moiety attached to an aglycone. The aglycone is a molecule that is bioactive in its free form but inert untill the glycoside bond is broken by water or enzymes. This mechanism allows the plant to defer the availability of the molecule to an appropriate time, similar to a safety lock on a gun.

Fungi causes important plant diseases specially in tropical regions, imposing the need for the permanent search and development of antimicrobial compounds.

More use of bactericides and fungicides like organomercurial, carbamates etc. have posed serious problems to human and environmental health, so search of natural biodegradable source of bactericides and fungicides have always been quest for the researchers for control of bacterial and fungal diseases of plants. Looking into the wealth of plants in Marathwada especially Nanded, it was thought proper to explore the available plant wealth for their efficacy of their antimicrobial potential. This could provide an alternative to the present day pollution problem. Research on a more sustainable and environmental friendly agriculture system is the need of the hour, as there is a growing concern on the deteriorating quality of the environment as a result of intensive agriculture. So with this view, the present investigation was undertaken to select plant extracts that could be effective in the development of new tools for the control of diseases caused by fungi to the plants of economic importance. Many plants contain active constituents that combine to give the plant its therapeutic value.

Preliminary screening of plants: After preliminary screening of ten angiospermic plants for their antimicrobial activity against fungus *Alternaria solani* by spore germination inhibition method only three out of ten plants were selected which showed maximum antifungal activity. These plants were h*emidesmus indicus* roots, family -Asclepediaceae), w*ithania somnifera* Roots (family - Solanaceae), and *Rauwolfia tetraphylla* Roots (family - Apocynaceae).

Material and Methods

Collection of plants: The plants used in the present studies were collected from different regions of Marathwada particularly Nanded. After pressing and drying herbarium sheets of these plants, their identification was confirmed through consultation with Department of Botany, Yeshwant Mahavidyalaya, Nanded using the "Flora of Marathwada". The leaves, rhizomes, tubers, and roots, of the selected plants were collected separately, surface sterilized with 0.1% $HgCl_2$ and washed two to three times with sterile distilled water. Plant parts like leaves, rhizomes, tubers, and roots, were separated and dried in a oven at 50-60 °C for 48 hours. Fine powders of these plant parts were prepared and preserved separately in polythene bags at room temperature (28 ± 2°C) for 48 hours.

Isolation of Phytopathogenic fungi: The test fungus namely *Alternaria solani,* was isolated from diseased leaves of tomato and *Fusarium moniliforme* was isolated from Maize seeds. For this the affected parts of the host were brought to the laboratory in polythene bags. They were cut into small pieces; surface sterilized with 0.1 per cent $HgC1_2$ solution and passed through three changes of sterile distilled water. The affected bits were placed aseptically on Glucose Nutrient Agar (GNA) plates. The fungal growth from the affected bits was picked up and transferred on GNA slalnt. The fungus identification was confirmed using manual of fungi maintained on GNA slants for further investigation.

***Fusarium moniliforme*, Deuteromycetes, Form Family-Tubercul ariaceae:** The fungus is pathogenic to plants especially in agricultural settings. The fungus causes serious diseases such as damping off of seedlings, root rot, wilting of several plants and rots of fruits and vegetables.

***Alternaria solani*, Deuteromycetes, Form Family-Dematiaeae:** *Alternaria solani* causes diseases of several crop plants of family solanaceae. The early symptoms are in the form of small spots on the leaves which later on enlarge to form concentric rings. The fungus also infects fruits and tubers in severe condition.

Preparation of plant extracts: For testing efficacy of plant extracts hot water, cold water, alcoholic (ethanolic) and ethyl acetate extracts of these plant parts were prepared. Hot water extract was prepared by heating extract in a container at 80 °C temperature for 20 minutes. 5 ml of alcohlic (ethanolic) and ethyl acetate extracts were evaporated on water bath and sterile distilled water was added to make up the volume of 5 ml. These extracts were used for further experiments

Plant Extracts: 2.5g / 5g / 7.5g / 10g powder each of the plant parts were suspended / mixed separately in 100ml sterilized distilled cold water, hot water, alcohol (ethanol) and ethyl acetate in 250 ml conical flasks. They were thoroughly shaken and then the conical flasks were allowed to stand for 12 hours at room temperature. The contents were filtered through Whatman filter paper no.1. The filtrates were used as 2.5 %, 5 %, 7.5 % and 10 % plant extracts respectively.

***In-vivo* testing of plant extract by seedling emergence method:** In order to evaluate the effect of plant extracts on percent seedling emergence, shoot length, root length, shoot rot and root rot the test seeds were soaked in 10 % plant extracts for 24 hrs. All 10% plant part extracts only was used for soaking seeds because these extracts exhibited antifungal properties in terms of percent inhibition of spore germination of test fungi, and 10% extracts showed maximum inhibition of spore germination and further dilutions showed decrease in inhibition of spore germination (By spore germination method).

These seeds were sown 2cm deep in earthen pots (25 cm diameter) filled with sterilized soil. After ten days percent seedling emergence, shoot length, root length, shoot root, root rot of each test seed was recorded. During the present studies, the test seed Maize var.local was assesed.

In order to record root length and shoot length seedlings were uprooted, washed in distilled water, placed on clear dry filter paper and measurements were taken using meter scale.

Results and Discussion

Effect of root extracts of *Hemidesmus indicus* (R.Br.) on Seedling emergence, Root and Shoot length, Shoot and Root rot of Maize variety local: To study effect of root extracts of *Hemidesmus indicus* on seedling emergence, shoot and root length, shoot and root rot of maize var local, an experiment was

set up. The test seeds were soaked in cold water, hot water, ethyl acetate and alcoholic root extracts for 24 hours. These soaked seeds were sown in earthen pots containing sterilized soil and grown for ten days at room temperature. On eleventh day seedling emergence, shoot and root lengths and root and shoot rots were recorded. Seeds soaked in sterile distilled water for 24 hrs served as control.

Table-1

Solvent extract (10%)	Seedling emergence (%)	Root length (cm)	Shoot length (cm)	Shoot Rot	Root rot
CW	53	3.6	4.2	+	+
HW	56	3.9	4.4	+	+
EA	69	4.3	5.6	-	-
AL	79	4.8	6.0	-	-
Control (SDW)	**45**	**3.2**	**4.0**	+	+

CW- Cold water, + = Low rot, HW- Hot water, ++ = Moderate rot, EA- Ethyl acetate , +++ = High rot, AL- Alcohol (ethanol), - = No rot, SDW- Sterile distiiled water.

Result: It is evident from results presented in table- 1 that, almost all the extracts were found to be supportive or stimulatory for seedling emergence, shoot and root length of the test seeds in more or less degree.

Maximum seedling emergence was recorded in alcoholic extract (79%) followed by ethyl acetate extract (69%, control 45%).

Maximum root and shoot lengths were recorded in alcoholic extracts followed by ethyl acetate extract (Root length- alcoholic extract 4.8 cm, ethyl acetate extract 4.3 cm, control 3.2 cm and shoot length alcoholic extract -6.0 cm, ethyl acetate extract 5.6 cm, control 4.0 cm).

Cold and hot water extract treated seeds have shown the shoot and root rots at par with control but alcoholic and ethyl acetate extract treated seeds did not show any rot.

Effect of roots extracts of *Withania somnifera* on Seedling emergence, Root and Shoot length, Shoot and Root rot of Maize variety local: To study effect of root extracts of *Withania somnifera* on seedling emergence, shoot and root length, shoot and root rot of maize var local, an experiment was set up. The test seeds were soaked in cold water, hot water, ethyl acetate and alcoholic root extracts for 24 hours. These soaked seeds were sown in earthen pots containing sterilized soil and grown for ten days at room temperature. On eleventh day seedling emergence, shoot and root lengths and root and shoot rots were recorded. Seeds soaked in sterile distilled water for 24 hrs served as control.

Table-2

Solvent extract (10%)	Seedling emergence (%)	Root length (cm)	Shoot length (cm)	Shoot rot	Roo t Rot
CW	48	3.4	4.2	+	+
HW	48	3.5	4.2	+	+
EA	56	3.7	4.5	-	-
AL	63	3.9	4.9	-	-
Control (SDW)	45	3.2	4.0	+	+

CW- Cold water, + = Low rot, HW- Hot water, ++ = Moderate rot, EA- Ethyl acetate, +++ = High rot, AL- Alcohol (ethanol), - = No rot, SDW- Sterile distiiled water

Result: It is evident from results presented in table-2 that, almost all the extracts were found to be supportive or stimulatory for seedling emergence, shoot and root length of the test seeds in more or less degree.

Maximum seedling emergence was recorded in alcoholic extract (63%) followed by ethyl acetate extract (56%, control 45%).

Maximum root and shoot lengths were recorded in alcoholic extracts followed by ethyl acetate extract (root length- alcoholic extract 3.9 cm, ethyl acetate extract 3.7 cm, control 3.2 cm and shoot length alcoholic extract -4.9 cm, ethyl acetate extract 4.5 cm, control 4.0 cm).

Seeds treated with cold and hot water extracts have shown the shoot and root rots at par with control but alcoholic and ethyl acetate extract treated seeds did not show any rot.

Effect of root extracts of *Rauwolfia tetraphylla* on Seedling emergence, Root and Shoot length, Shoot and Root rot of Maize variety local: To study effect of root extracts of *Rauwolfia tetraphylla* on seedling emergence, shoot and root length, shoot and root rot of maize var local, an experiment was set up. The test seeds were soaked in cold water, hot water, ethyl acetate and alcoholic root extracts for 24 hours. These soaked seeds were sown in earthen pots containing sterilized soil and grown for ten days at room temperature. On eleventh day seedling emergence, shoot and root lengths and root and shoot rots were recorded. Seeds soaked in sterile distilled water for 24 hrs served as control.

Table-3

Solvent extract (10%)	Maize				
	Seedling emergence (%)	Root length (cm)	Shoot length (cm)	Shoot rot	Root rot
CW	47	3.3	4.2	+	+
HW	48	3.5	4.4	+	+
EA	52	3.9	4.9	-	-
AL	59	4.2	5.1	-	-
Control (SDW)	**45**	**3.2**	**4.0**	+	+

CW- Cold water, + = Low rot, HW- Hot water, ++ = Moderate rot, EA- Ethyl acetate, +++ = High rot, AL- Alcohol (ethanol), - = No rot, SDW- Sterile distiiled water.

Result: It is evident from results presented in table- 3 that, almost all the extracts were found to be supportive or stimulatory for seedling emergence, shoot and root length of the test seeds in more or less degree.

Maximum seedling emergence was recorded in alcoholic extract (59%) followed by ethyl acetate extract (52%, control 45%).

Maximum root and shoot lengths were recorded in alcoholic extracts followed by ethyl acetate extract (root length- alcoholic extract 4.2 cm, ethyl acetate extract 3.9 cm, control 3.2 cm and shoot length alcoholic extract -5.1 cm, ethyl acetate extract 4.9 cm, control 4.0 cm).

Seeds treated with cold and hot water extracts have shown the shoot and root rots at par with control but alcoholic and ethyl acetate extract treated seeds did not show any rot.

Conclusion

The plant part extracts stimulated seedling emergence, root and shoot elongation of Maize var. local of all the plants, *Hemidesmus indicus* plant extracts were found to be more effective against seed borne pathogens compared to other plant extracts. Out of the four solvent extracts viz. cold water, hot water, ethyl acetate, alcohol(ethanol), alcoholic extracts of all plants showed more stimulation. In general seeds soaked in plant extracts of *Hemidesmus indicus*, *Withania somnifera*, and *Rauwolfia tetraphylla* showed maximum seed germination, root and shoot elongation and seedling emergence. *Hemidesmus indicus* showed higher seedling emergence, root and shoot elongation of Maize seed which have not been recorded earlier by any worker.

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