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Determination of Compatibility of Fungitoxicants and de-oiled cakes with Bioagent in wheat rhizosphere

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Abstract: Importance of wheat (*Triticum aestivum* L.) as staple food is well known as nearly 35% of the world population depends on wheat and demand for wheat is expected to grow faster than any other major crop. It provides about 20 percent of the total food calories for the human being. Wheat is grown primarily for the grain which is ground and utilized in the form of flour called atta or whole-meal for the manufacturing various kinds of breads, cakes, cookies, crackers, breakfast, cereals etc. The wheat producing states in India are Bihar, Gujarat, Haryana, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Uttar Pradesh, Uttarakhand and West Bengal. In Uttar Pradesh area under wheat is 9.65 million hectares with total production of 26.87 million tones with productivity of 2786 kg ha^{-1}. Productivity of wheat in Uttar Pradesh is at par with the country but far behind than potential wheat cultivating states *i.e* Haryana and Punjab. Estimated productivity of Punjab is 4596 kg/ha during 2015-16 which is 1810 kg ha^{-1} higher than that of Uttar Pradesh for the same period (Anonymous 2016). In recent past, several bioagents including *Trichoderma harzianum* and *Pseudomonas fluorescens* have been found to minimize the effect of moisture stress in several crops; hence it is assumed that these bioagents may be helpful in mitigating certain physiological stress. However the agrochemicals often being used in wheat cultivation also must be tested for their compatibility with these bioagents.

MATERIALS AND METHODS

Glassware

For laboratory experiments Corning and Borosil made glasswares were used. The glassware already used, were first cleaned and dipped in potassium dichromate + sulphuric acid solution (Potassium dichromate 200g, concentration sulphuric acid 1200 ml + distilled water 800 ml) overnight and then washed with lablet followed by tap water and finally rinsed with distilled water and dried properly to make them moisture free.

Sterilization

All the glasswares were sterilized in hot air oven at 180°C for one hour. The solid media and distilled water were autoclaved at 1.1 kg/cm^2 pressure for 15 minutes at 121.6^0 C .

Preparation of Media

Potato Dextrose Agar (PDA) and Broth, King's (B) Medium (KB) Agar and broth and *Trichoderma* selective medium (TSM) were used to multiply the bioagents . Composition of above mentioned media have been given below.

Potato Dextrose Agar (PDA)

Ingradients used

Pealed potato	: 200g
Dextrose	: 20g
Agar agar	: 20g
Distilled water	: 1000 ml

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Final volume was made to 1000 ml using distilled water

Potato Dextrose Broth (PDB)

Ingredients used

Potato inclusion	: 200gm
Dextrose	: 20 gm
Distilled water	: 1000 ml

Final volume was made to 1000 ml using distilled water

Preparation of PDA Media

About 250 g of potatoes was peeled and cut into fine sliced pieces. Two hundred g of potato slices were weighed and placed into a stainless steel pan. About 1000 ml of water was added to potato pieces and boiled gently for such a period until they are easily mashed by a glass rod. The decoction thus obtained was filtered through muslin cloth and all the liquid was squeezed out in a measuring cylinder and potato pieces were discarded. Now sufficient amount of boiled water was added to make the volume 1000 ml. Now pre-weighed 20 g agar-agar was added (20 g) slowly to the boiling solution so as to dissolve it. At the same time (20g) dextrose was also added in boiling solution (melted with agar) and final volume maintained to one litre. While boiling the solution was steered with glass rod to mix the agar-agar and dextrose properly. It was poured @ about 200 ml in each of four conical flasks of 500 ml capacity and 10 ml per culture tube to prepare the PDA slants. Flasks and culture tubes both were tightly plugged with non-absorbant cotton and wrapped with butter paper and rubber bands. The culture tubes and flasks were placed vertically (mouth up) in wire baskets and then autoclaved at 1.1 kg/ cm^2 pressure for 20 minutes at 121.6 ^{0} C and cooled before pouring in to Perti plates.

Preparation of TSM

All the ingredients were mixed with distilled water; agar was added to it in a stainless steel pan and stirred with glass rod for proper mixing. Now the medium was filtered through a muslin cloth by squeezing out whole liquid. 200 ml dissolved medium was placed in each 500 ml capacity flask. Flasks were tightly plugged with non-absorbent cotton and wrapped with butted paper and rubber band. Medium was autoclaved at 1.1 kg/cm^2 pressure for 20 minutes (121.6^{0}C) and cooled before pouring in to Petri plates.

Preparation of King's(B) medium

Medium was prepared following the method used for preparing TSM as given in point no (3.6.2). The Bacteria initially isolated in a pure culture on King's B medium and sub cultured on PDA slants. After initial growth of *P. fluorescens* on King's B medium, identity of the bacterium was confirmed following standard. After proper growth , bacteria culture was stored in a refrigerator for long term storage, so as to be used in further studies.

Compatibility of *Trichoderma harzianum* with different fungicides

Determination of compatibility of *Trichoderma harzianum* with different fungicides *in- vitro*

Four fungicides were tested *in vitro* for their compatibility with *Trichoderma harzianum* using poisoned food technique. Required amount of fungicides were added in each 250 ml capacity flasks, containing 100 ml sterilized PDA media to obtain 80 ppm , 90 ppm and 100 ppm concentration of each test fungitoxicant. It was mixed thoroughly by shaking the flask prior to pouring in sterilized Petri Plates. The medium was allowed to solidify over night and then 5 mm disc from seven days old actively growing culture of *Trichoderma harzianum* was placed in centre of each Petri Plates. PDA medium mixed with distilled water only serve as check. Three replications were maintained for each treatments. Inoculated plates were incubated

at 28± 2° C in BOD incubator. Observation were recorded on radial growth of *Trichoderma harzianum* at an interval of each 24 hr's, up to 4 days. Percent inhibition was calculated using following formula.

$$\text{Percent inhibition} = \frac{C - T}{C} \times 100$$

Where :

C = Growth of fungus in control.

T = Growth of fungus in Treatment.

Experimental details

Biocontrol agent used - *Trichoderma harzianum* (SV-03)

Treatments (Fungicides) and their different conc. - 13

Replication - 03

Doses - 80ppm, 90ppm, 100ppm

Design-Completely Randomized Design (CRD)

Table 3.2: Treatments details

S. No.	*Treatments details*	*Dose (ppm)*
T_1	Penflufen (Emesto)	80ppm
T_2	Penflufen (Emesto)	90ppm
T_3	Penflufen (Emesto)	100ppm
T_4	Pencycuron (Monocern)	80ppm
T_5	Pencycuron (Monocern)	90ppm
T_6	Pencycuron (Monocern)	100ppm
T_7	Cymoxanil (Curazate)	80ppm
T_8	Cymoxanil (Curazate)	90ppm
T_9	Cymoxanil (Curazate)	100ppm
T_{10}	Prolifer (Fluopicolide)	80ppm
T_{11}	Prolifer (Fluopicolide)	90ppm
T_{12}	Prolifer (Fluopicolide)	100ppm
T_{13}	Control	80ppm, 90ppm, 100ppm

Determination of self compatibility of bioagents, de-oiled cakes, and fungitoxicants in wheat rhizosphere

Experimental details-

Biocontrol agent used-*Trichoderma harzianum* and *Pseudomonas fluorescens*

Substrate used-De-oiled cakes (Mustard cakes, Groundnut cakes, Cotton cakes, Mahua cakes)

Table 3: Treatment details

S. No.	*Treatments details (De-oiled cakes , Fungicides and Bioagents and their combination)*	*Rate of applicaton*
T_1	Soil application of Mustard de-oiled cakes	200 kg/ha
T_2	Soil application of Ground nut de-oiled cakes	200 kg/ha
T_3	Soil application of Cotton de-oiled cakes	200 kg/ha
T_4	Soil application of Mahua de-oiled cakes	200 kg/ha
T_5	Seed treatment with *Trichoderma harzianum*(SV-08)	10 gm/kg seed
T_6	Seed treatment with Penflufen(Emesto)	2.5 gm/kg seed
T_7	Seed treatment with Pencycuron (Monocern)	2.5 gm/kg seed
T_8	Seed treatment with Cymoxanil (Curazate)	2.5 gm/kg seed
T_9	Seed treatment with Prolifer(Fluopicolide)	2.5 gm/kg seed
T_{10}	Soil application of Mustard de-oiled cakes mixed with *Trichoderma harzianum* (SV-26)	Cake200kg/ha& Bioagent
T_{11}	Soil application of Cotton de-oiled cakes mixed with *Trichoderma harzianum*(IRRI-1)	Cake200kg/ha& Bioagent
T_{12}	Soil application of Ground nut de-oiled cakes mixed with *Trichoderma harzianum*(SV-3)	Cake200kg/ha& Bioagent
T_{13}	Soil application of Mahua de-oiled cakes mixed with *Trichoderma harzianum*(SV-30)	Cake200kg/ha& Bioagent
T_{14}	Soil application of Mustard de-oiled cakes mixed with *Pseudomonas fluorescens*(PF-28)	Cake200kg/ha& Bioagent
T_{15}	Soil application of Cotton de-oiled cakes mixed with *Pseudomonas fluorescens*(PF-6)	Cake200kg/ha& Bioagent
T_{16}	Soil application of Ground nut de-oiled cakes mixed with *Pseudomonas fluorescens*(PF-2)	Cake200kg/ha& Bioagent
T_{17}	Soil application of Mahua de-oiled cakes mixed with *Pseudomonas fluorescens*(PF-4)	Cake200kg/ha& Bioagent
T_{18}	Seed treatment with Penflufen (Emesto)+ *Trichoderma harzianum*(SV-26)	Chemical2.5gm/kg& Bioagent-10gm/kg seed
T_{19}	Seed treatment with Pencycuron (Monocern)+ *Trichoderma harzianum*(IRRI-1)	Chemical 2.5gm/kg & Bioagent-10gm/kg seed
T_{20}	Seed treatment with Cymoxanil (Curazate) + *Trichoderma harzianum*(SV-30)	Chemical 2.5gm/kg & Bioagent-10gm/kg seed

S. No.	Treatments details (De-oiled cakes , Fungicides and Bioagents and their combination)	Rate of applicaton
T_{21}	Seed treatment with Prolifer (Fluopicolide)+ *Trichoderma harzianum*(SV-3)	Chemical 2.5gm/kg & Bioagent-10gm/kg seed
T_{22}	Seed treatment with Penflufen (Amisto)+ *Pseudomonas fluorescens*(PF-4)	Chemical 2.5gm/kg & Bioagent-10gm/kg seed
T_{23}	Seed treatment with Pencycuron (Monocern)+*Pseudomonas fluorescens*(PF-28)	Chemical 2.5gm/kg & Bioagent-10gm/kg seed
T_{24}	Seed treatment with Cymoxanil (Curazate)+ *Pseudomonas fluorescens*(PF-6)	Chemical 2.5gm/kg & Bioagent-10gm/kg seed
T_{25}	Seed treatment with Prolifer (Fluopicolide)+ *Pseudomonas fluorescens*(PF-2)	Chemical 2.5gm/kg & Bioagent-10gm/kg seed
T_{26}	Untreated seed	

Procedure of taking observations

Observations on shoot length, root length, fresh weight of root and dry weight of root etc. were recorded on randomly selected three plants from each pot in each replication of every treatments at stem elongation / jointing stage. These plants were uprooted and washed with tap water. The data were recorded on following parameters:

Root and shoot length

Root and shoot length were measured with the help of a measuring scale by selecting three plants randomly from each pot in each replication of every treatments at stem elongation/ jointing stage at 20 days of drought exposure same uprooted plants were also used for measuring fresh weight and dry weight of roots. Fresh weight and dry weight of roots in every treatment were also measured at 20 days of drought exposure before flowering.

$$\text{Percent Increase} = \frac{\text{Root length in treated plant} - \text{Root length in untreated plant}}{\text{Root length in untreated plant}} \times 100$$

$$\text{Percent Increase} = \frac{\text{Shoot length in treated plant} - \text{Shoot length in untreated plant}}{\text{Shoot length in untreated plant}} \times 100$$

Physio-biochemical observations

In addition to morphological observation, physiological and biochemical observation were also recorded from the wheat plants where drought exposure was provide at 90 days after sowing. Following physiological observation were recorded.

Leaf area (cm^2)

Total leaf area (cm^2) of wheat crop was measured at 90 days after sowing according to Quarrie and Jones equation(Aldesuquy *et al.*, 2014) as given below:

Leaf area (cm^2) = L×W ×F

Where,

L= Maximum length(cm)

W= Maximum width(cm)

F= Factor (0.75)

Flag leaf area (cm^2)

Flag leaf area (cm^2) Length and width of flag leaf area of each guarded plant was measured in centimeters at 90 days after sowing and then multiplied with 0.74 to get flag leaf area according to following formula of Muller (1991).

Flag leaf area (cm^2) = L×W ×F

Where,

L= Maximum length(cm)

W= Maximum width(cm)

F= Factor (0.74)

Relative water content (RWC)

The relative water content in recently matured leaves was determined following methods suggested by Brass and Wheaherly (1962). Ten discs from the completely extended leaves were excised and fresh weight was recorded. The weighed leaf discs were allowed to float on distilled water in a Petri dish for four hours and turgid weight was recorded. These leaf discs were then dried in a hot air oven at 70

^{0} C for 2-3 days until constant weight was achieved . Finally the dry weight of sample was recorded. The relative water content was estimated using the following formula :

$$RWC = \frac{FW-DW}{TW-DW} \times 100$$

Where,

FW-Fresh leaf weight

DW- Oven dry leaf weight

TW- Turgid leaf weight

Total chlorophyll content

The SPAD (Soil Plant Analytical Development) chlorophyll meter [(Minolta ™)]portable chlorophyll meter was used to acquire a rapid estimation of leaf chlorophyll content (Chelah *et al.,*2011). The measurement reading were taken on the upper-most colored leaf and five reading per leaf were taken from each plot and mean value were calculated. The measurement was done at around 11 am to 12 noon to avoid water content droplets on leaf surface.

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