PHYTOTOXKIT

Test procedure

1

RAPID METHOD FOR DETERMINATION OF THE WATER HOLDING CAPACITY (WHC) OF TEST SOILS

2

SIEVE AIR-DRIED SOIL THROUGH A SIEVE WITH A 2MM MESH TO ELIMINATE ALL COARSE MATERIAL

3

- FILL A GRADUATED CYLINDER TO THE 50 ML MARK WITH DISTILLED WATER

- FILL A 100 ML BEAKER WITH 90 ML SIEVED SOIL

4

POUR THE 50 ML WATER IN THE BEAKER WITH THE SOIL

5

MIX THE WATER THOROUGHLY WITH THE SOIL TILL THE SOIL IS COMPLETELY WATER SATURATED

6

WAIT FOR THE SOIL/WATER MIXTURE TO REACH EQUILIBRUM, LEADING TO A WATER SATURATED SOIL PHASE AND A LAYER OF WATER ON TOP

7

CAREFULLY POUR THE SUPERNATANT IN A GRADUATED 50 ML CYLINDER, TAKING CARE NOT TO CARRY OVER (SOLID) SOIL PARTICLES

8

WAIT FOR A FEW MINUTES AND POUR AGAIN THE WATER ON TOP OF THE SOIL IN THE GRADUATED CYLINDER

9

CALCULATE THE VOLUME OF WATER (Vsat) NEEDED FOR COMPLETE HYDRATATION OF THE TEST SOIL.

THIS VOLUME IS EQUIVALENT TO THE VOLUME OF WATER THAT HAS BEEN ADDED TO THE SOIL (= 50 ML) MINUS THE VOLUME OF SUPERNATANT WATER (S) RECOVERED IN THE GRADUATED CYLINDER

$$Vsat\ (ML) = 50 - S$$

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ADDITION OF REFERENCE SOIL AND TEST SOIL TO THE TEST PLATES AND HYDRATATION OF THE SOILS

1. CONTROL SOIL

11

POUR THE CONTENTS OF ONE BAG WITH REFERENCE SOIL (90 ML) IN THE LOWER COMPARTMENT OF A TEST PLATE

12

- TAKE A 50 ML SYRINGE AND FILL IT TO THE 35 ML MARK WITH DISTILLED WATER (35 ML is the volume of water giving 100% water saturation of 90 ML reference soil)

- EMPTY THE CONTENTS OF THE SYRINGE BY DROPPING THE WATER SLOWLY OVER THE WHOLE SURFACE OF THE SOIL IN THE TEST PLATE

13

- WAIT A FEW MOMENTS FOR THE WATER TO HYDRATE THE SOIL COMPLETELY
- WITH THE AID OF A SPATULA, SPREAD THE WET SOIL EVENLY OVER THE TOTAL SURFACE OF THE BOTTOM COMPARTMENT OF THE TEST PLATE

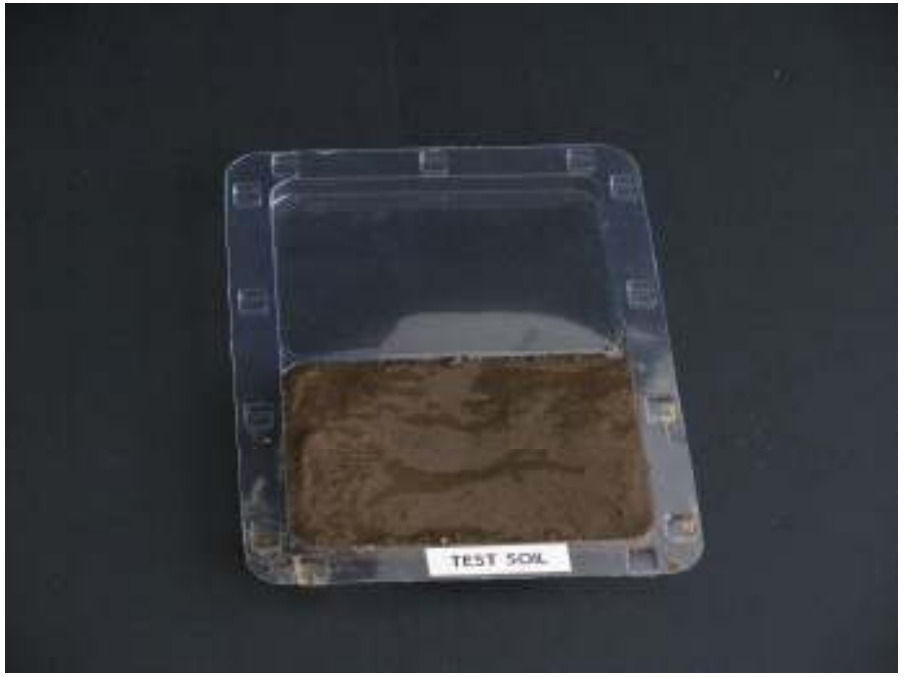
14

FLATTEN THE SURFACE OF THE SOIL TO OBTAIN A LAYER OF UNIFORM DEPTH

15

2. TEST SOIL

- FILL A 100 ML BEAKER TO THE 90 ML MARK WITH THE SIEVED TEST SOIL
- TRANSFER THIS VOLUME OF SOIL TO THE BOTTOM COMPARTMENT OF A TEST PLATE



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- HYDRATE THE TEST SOIL WITH A VOLUME OF WATER EQUAL TO Vsat (see PICTURE 9)

- PROCEED FURTHER AS INDICATED IN PICTURES 12 TO 14 TO FLATTEN THE SURFACE OF THE SOIL

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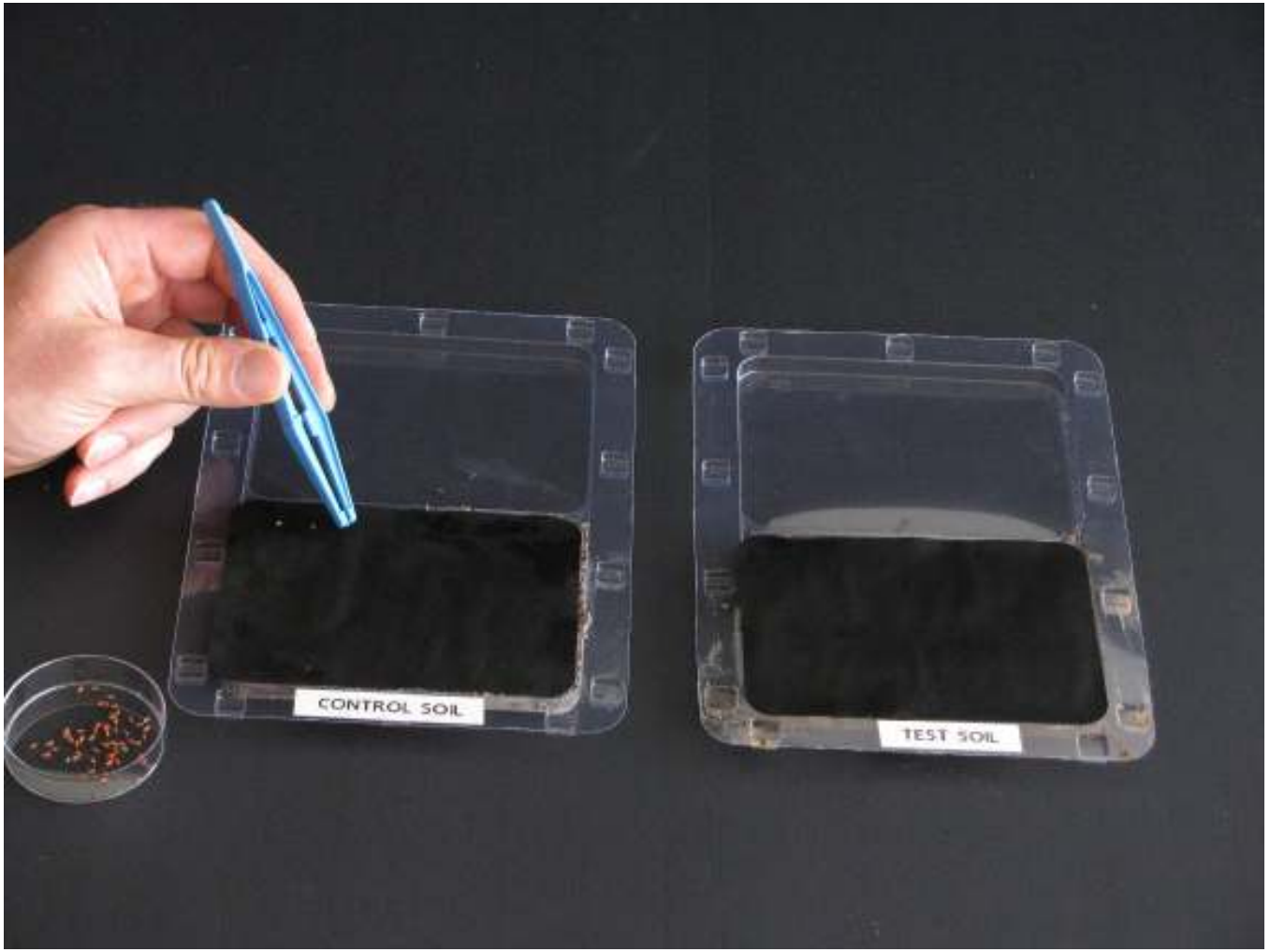
PLACING OF THE FILTER PAPER AND SEEDS IN THE TEST PLATE

1. PLACING OF THE FILTER PAPER

18

PUT A BLACK FILTER ON TOP OF THE HYDRATED (CONTROL AND TEST) SOILS IN ALL THE TEST PLATES AND WAIT UNTIL THE FILTER IS COMPLETELY WET



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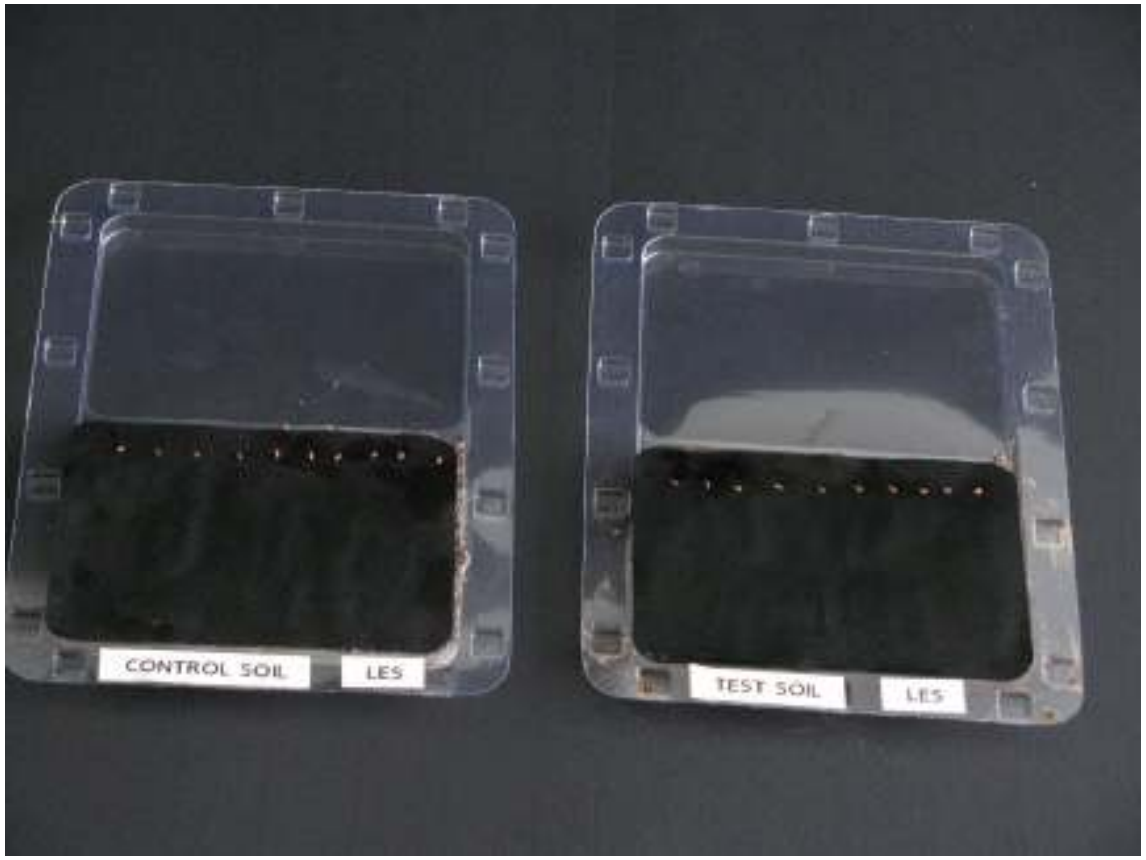
2. PLACING OF THE SEEDS



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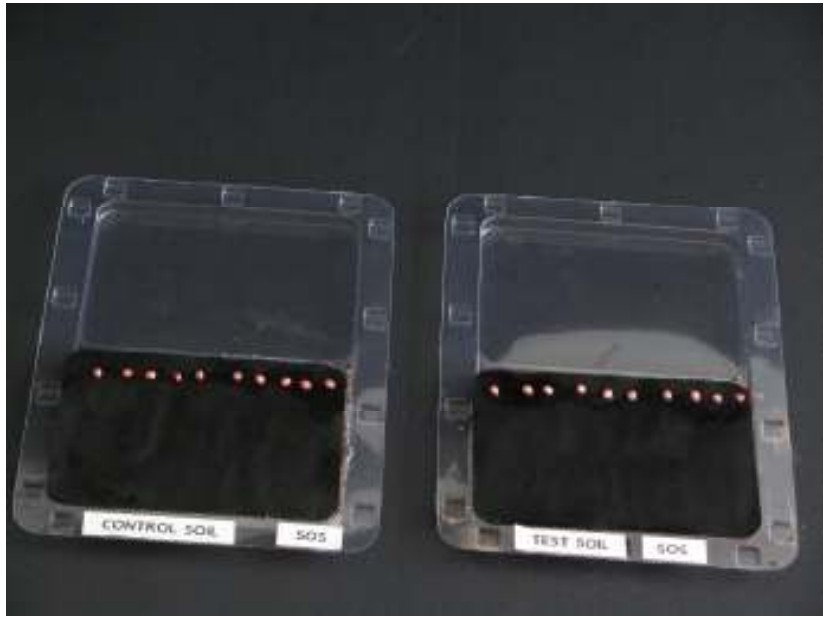
- PLACE 10 SEEDS OF THE SAME TEST PLANT ON TOP OF THE FILTER PAPER (IN ONE ROW AND AT EQUAL DISTANCE OF EACH OTHER).

-THE SEEDS SHALL BE PLACED NEAR THE TOP OF THE FILTER PAPER, (AT ABOUT 1 CM OF THE MIDDLE RIDGE OF THE TEST PLATE)



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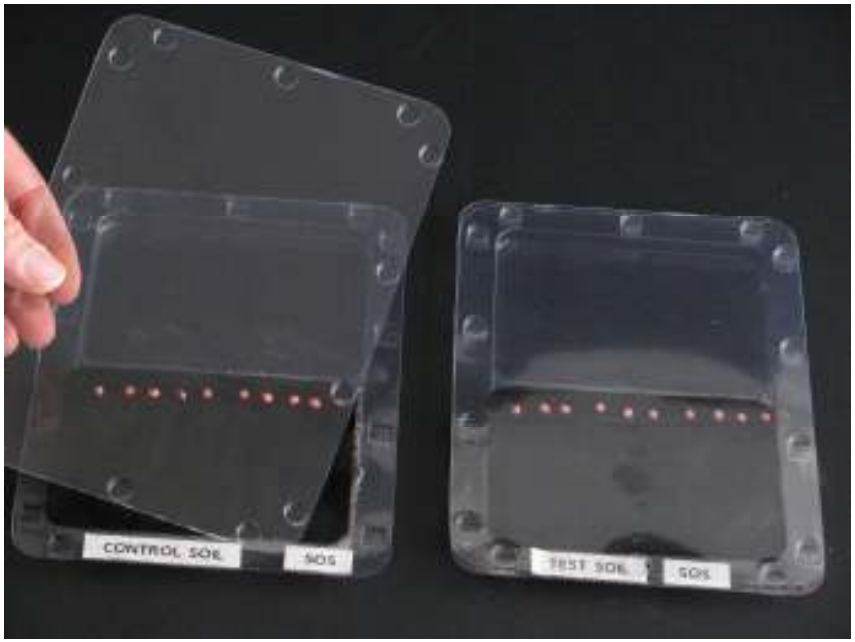
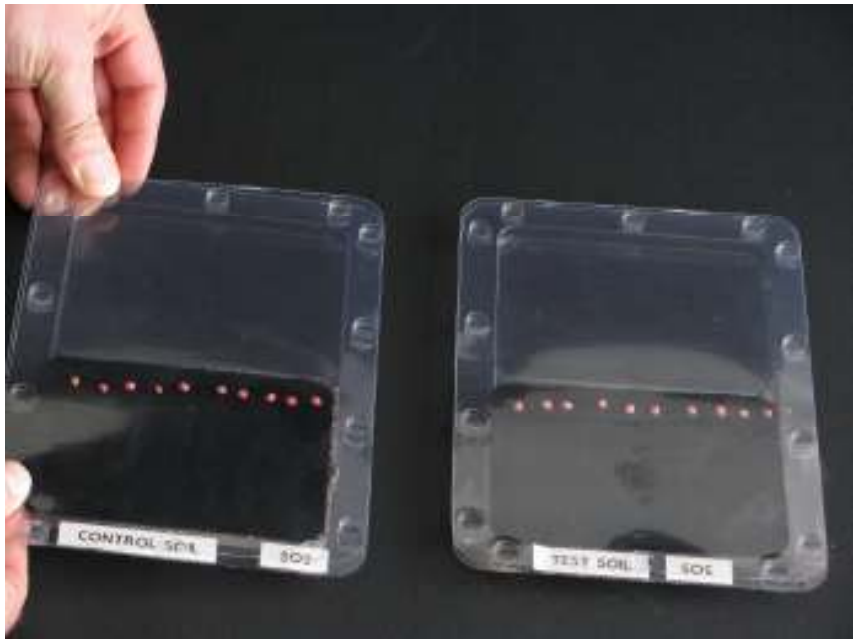
PHYTOTOXKIT TESTS ARE NORMALLY CARRIED OUT IN 3 REPLICATES WITH 3 DIFFERENT SEEDS :

* THE MONOCOTYL SORGHO (*Sorghum saccharatum*) *(*SOS)
* THE DICOTYL GARDEN CRESS (*Lepidium sativum*) (LES)
* THE DICOTYL MUSTARD (*Sinapis alba*) (SIA)





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3. CLOSING OF THE TEST PLATES

CAREFULLY PLACE THE COVER ON THE TEST PLATE AND CLICK THE PROTRUDING PARTS INTO THE CORRESPONDING HOLES OF THE BOTTOM PART

23

INCUBATION OF THE TEST PLATES

PUT THE TEST PLATES VERTICALLY IN THE CARDBOARD HOLDERS (6 plates per holder)

24

PUT THE CARDBOARD HOLDERS WITH THE TEST PLATES IN THE INCUBATOR AND INCUBATE AT 25° C, FOR 3 DAYS, IN DARKNESS

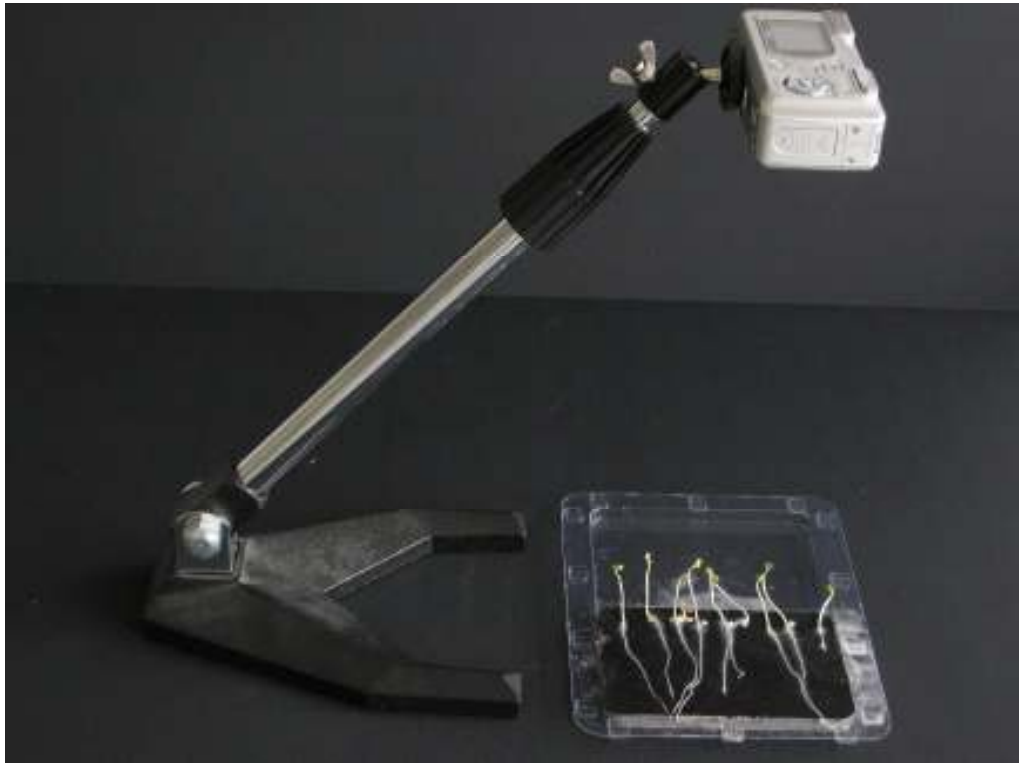

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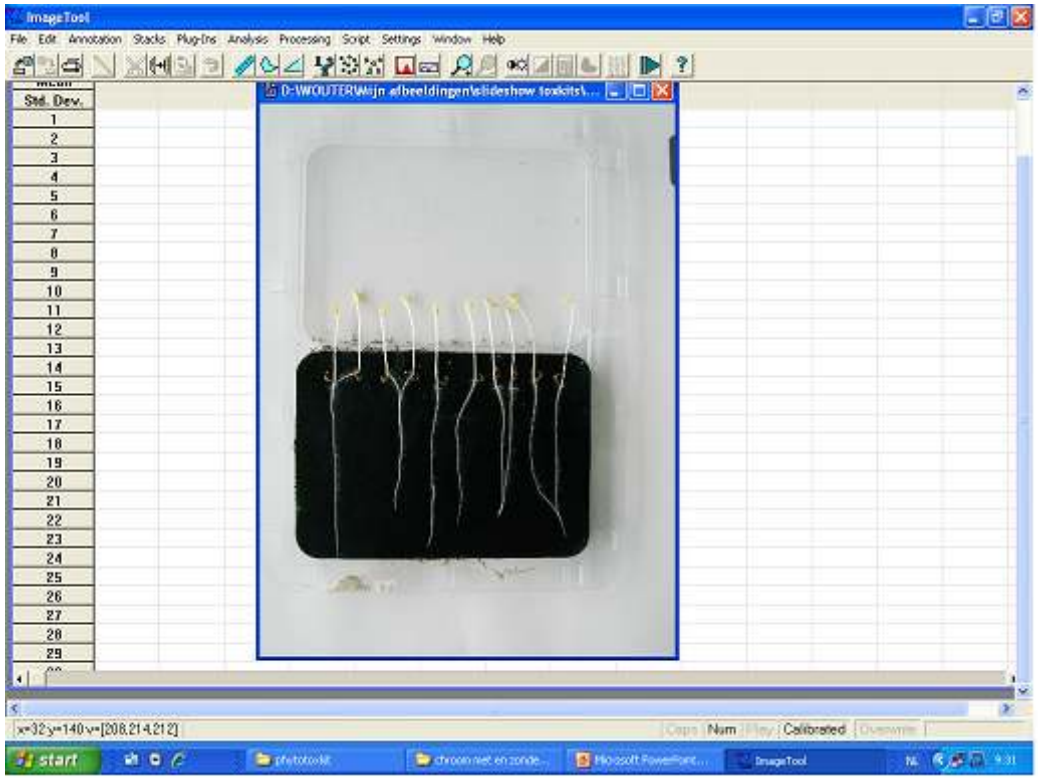
IMAGE RECORDING AT THE END OF THE EXPOSURE PERIOD

- TAKE THE TEST PLATES OUT OF THEIR HOLDER
- PUT THE PLATES ON A HORIZONTAL SURFACE
- TAKE A PICTURE OF EACH PLATE (e.g. WITH A DIGITAL CAMERA)
- TRANSFER THE PICTURES TO A COMPUTER FILE



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ANALYSIS AND MEASUREMENTS

- MEASURE THE LENGTHS OF THE ROOTS (AND THE SHOOTS)
  WITH AN APPROPRIATE IMAGE ANALYSIS PROGRAM
- PERFORM THE PRESCRIBED DATA TREATMENT TO CALCULATE THE PERCENTAGE
  SEED GERMINATION AND GROWTH INHIBITION OF THE PLANTS IN THE TEST SOIL
  VERSUS THE REFERENCE SOIL