THE CONTROL OF SEEDLING DISEASES OF
ALFALFA AND SUGAR BEET

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Damping - off diseases

Khan

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ABSTRACT

Field experiments, greenhouse and laboratory tests were conducted during 1963 - 64 in studies with the control of damping - off diseases in alfalfa and sugar beets.

The effectiveness of Ceresan, Ceredon, Ceredon special, Rhizoctol, Orthocide 50%, Rhizoctol combination, Spergon and T.M.T.D,, applied at three rates,was tested under greenhouse and field moist soil conditions on both the crops.

Under greenhouse conditions alfalfa responded consistantly to seed treatment chemicals with all the dosages but excess treatment was the most effective. Orthocide 50% and Ceredon group were the best of the fungicides tested.

Field tests made at AREC did not indicate any benefits from fungicidal seed treatment of alfalfa.

With the exception of Orthocide and T.M.T.D, chemical seed treatment impaired significantly the germinability of sugar beet seeds with all dosages.

Both under greenhouse and field conditions, in most cases seed treatment with 0.2% increased the stand of sugar beet seedlings significantly above the check but Orthocide, Rhizoctol and T.M.T.D were also effective with excess treatment.

Soil steaming prevented completely the damping - off of alfalfa seedlings.

All the isolates were found extremely pathogenic on alfalfa seedlings under moist soil conditions.

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INTRODUCTION

Damping - off has been recognized as a definite disease of various crops for more than a century but it is doubtful if even today many growers are aware of its true nature or prevalence.

Growers generally regard poor stands as an indication of poor seed germination or low viability, and in order to offset these poor stands they usually plant much more seed than would be required, if the seedlings remained healthy. Also sometimes replanting is necessary. Besides the added cost of extra seed used, labour expenses also increase for replanting and/or thinning operation if damping - off is not as serious as expected.

Much work has been done on various phases of damping - off and as a result a voluminous literature on the subject has appeared. The outstanding feature of the many studies is the decided variation among the results obtained. This fact serves to emphasize the complex nature of the problem. It has also created the problem of selecting satisfactory control measures for a given locality. It is not likely that many control methods which have been evolved are equally efficient and practical. The variable performance of any particular treatment under a variety of soil and environmental conditions further complicates the general problem. Also it is evident that the many different fungi and host plants may require individual control adaptations.

These and similar facts and considerations are largely responsible for this attempt to evaluate and clarify the damping - off problem as it exists in Lebanon. Another objective of this study is to test farm and greenhouse soil for damping - off organisms.

Numerous investigations have been made into the causes of losses due to poor stands, caused by various seed-borne and soil-borne organisms. For which different methods of control may be employed either through soil treatments or seed treatments.

A good number of definite experimental data have been forthcoming to substantiate the value of such treatments in increasing stands and improving germination of field legumes especially alfalfa (Medicago sativa L.), because natural limitations of seed production in this crop has increased the cost of its seed

Sugar beet (Beta vulgaris L.) a recently introduced crop in Lebanon, is also subject to poor stands due to damping - off diseases. Seed balls of sugarbeet producing 2 to 5 seedlings, may compensate the poor stand but the surviving seedlings may be too close to allow their development to the maximum sizes of their roots. This can be eliminated by sowing the so called "monogerm seed" but here the danger of losing seedlings due to damping - off is even more serious.

Therefore preventive control measures should be practised, for which this study, conducted under greenhouse and field conditions, reports the results for the effectiveness of seed-treatments for the control of damping - off in comparison with soil disinfection through steaming.

REVIEW OF LITERATURE

1. DAMPING - OFF CAUSED BY FUNGI:

Many fungi are known to be capable of causing damping - off diseases in one or all of its several aspects but *Pythium* spp. are the most common ones.

HALPIN *et al* (18, 19) in 1954, followed by DICKSON (12) in 1956, tested *Pythium* spp. for their relative pathogenicity on alfalfa, red clover and lespedeza under greenhouse conditions in pure sand culture at different soil temperatures (16, 20, 24 and 28°C). *Pythium debaryanum*, Hesse, *Pythium irregulare* Buisman, *Pythium splendens* Braun and *Pythium ultimum* Trow. were the most pathogenic on each host under all conditions. *Pythium arrhenomanes* Drechsl, *Pythium graminicola* Subrm and *Pythium paroecandrum* Drechsl, were relatively non-pathogenic on these legumes, while *Pythium rostratum* Butler. was completely nonpathogenic. WELTZIEN (40) identified the isolates obtained from damped - off seedlings of alfalfa, as *Pythium ultimum* and *Pythium debaryanum*.

CORMACK (10) reported from the greenhouse, laboratory and field experiments that *Ascochyta imperfecta* PK. parasitized the seedlings of alfalfa, especially in cool moist conditions and caused post - emergence damping - off which was controlled effectively by seed treatment with new improved Ceresan and Arasan. From pathogenicity tests with *Leptodiscus terristris* Gerdemann; on small seeded legumes in greenhouse, GERDEMANN (16) found that all the legumes used were susceptible to this pathogen which caused damping - off but alfalfa was the most susceptible one.

Isolates of Phoma trifolii E.M. Johnson and Valleau and Phoma herbarum var.medicaginis West. ex Rab. were tested in the greenhouse by SCHENCK and GERDEMANN (36), for their pathogenicity on alfalfa and redclover in separate pots. No significant difference between emergence of seedlings inoculated with either pathogen was found, indicating that the pre - emergence damping - off was the same for both the pathogens. The noninoculated control seedlings remained healthy. Isolates were significantly more pathogenic on the host from which they were obtained.

PETERS (cited by BUCHHOLTZ 6.) reported that Pythium debaryanum and Phoma betae (Oud.) Fr. have been the chief isolates from damped - off seedlings of sugar beets but very few isolates of Rhizoctonia spp. were obtained. Aphanomyces levis deBary.also invaded young seedlings of sugar beets in a manner similar to that of Pythium debaryanum but acted more slowly and therefore produced less severe damage. According to WRIGHT (43), Pythium ultimum and several strains of Rhizoctonia solani Kuehn. were found directly pathogenic to a good number of broad leaved vegetable plants and also of agronomic plants. He also indicated that in addition to these pathogens Fusarium spp. caused fairly heavy post - emergence damping - off in sugar beets when planted in inoculated soil.

POUND (35) isolated the same above mentioned organisms from sugar beets in Washington State but these differ in importance according to the locality concerned. Pythium spp. are chiefly responsible for pre-emergence damping - off, usually during the first two weeks of seedling growth. Phoma may also cause pre-emergence damping - off but Rhizoctonia spp. attack slightly older seedlings. Aphanomyces attacks seedlings as

Pythium does but it seldom causes pre - emergence damping - off. BUTLER (7) isolated the same fungi from sugar beets in South West England. He also mentioned that besides these fungi certain strains of Rhizoctonia solani in North America and some strains of Alternaria solani Ell & G. Martin, in Germany were found associated too.

2. FACTORS AFFECTING THE SEVERITY OF DAMPING- OFF :

a. TEMPERATURE

GERDEMANN (15) studied the effectiveness of Arasan on red clover seed treatment in greenhouse in warm (70 to 75^{o}F) and cool (45 to 55^{o}F) soils which were infested with Pythium isolates. Moisture was kept high in all tests. Damping - off was much more serious in warm than in cool soil. Treatment of seed with 0.5% Arasan gave a low degree of control in warm soil but also did not increase seedlings emergence in cool soil above that of check.

GRAHAM et al (17) conducted experiments to determine the effect of different temperatures on damping - off of Ladino clover and Lespedeza, caused by Fusarium roseum (Cke) Snyder & Hansen, Pythium debaryanum and Rhizoctonia solani, at three different levels of soil moisture. The most favourable temperature for mycelial growth of both Fusarium and Rhizoctonia was 66 to 89^{o}F, while for Pythium debaryanum it was 68 to 87^{o}F. In general optimum temperature for most rapid pre - emergence damping - off of clovers by these three fungi was from 80 to 94^{o}F and mostly post - emergence damping - off occurred at 59 to 73^{o}F. Fusarium was most active in moderatly dry soil while Pythium did so in wet soil. Rhizoctonia was little affected by soil moisture. From unpublished results obtained at Cornell. Agric. Expt. Sta. TYLER (37) has described likewise that seed rot and damping - off in alfalfa are likely to be more severe in warm soils than in cool ones. Seed treatment with Arasan did not control damping - off in warm soils but gave poor to fair control of damping - off in cool soils.

In the greenhouse HANSON (21) studied the effect of different temperatures (16, 20 and 28°C) on damping - off and the response to seed treatments with Arasan 50%, Orthocide 75%, Phygon 50% (50% dichlone) and Vancide 51 (30% sodium dimethyl dithiocarbonate and sodium derivative of 2-mercapto benzothizole) on stands of forage legumes at emergence and 15 days after emergence in a naturally infested Carlisle muck soil. Seed treatment was very effective in reducing pre - emergence killing but did not control the post - emergence damping - off. All fungicides used improved the stands at all levels of temperature. There was no significant difference among fungicides used on red clover, while on alfalfa Orthocide proved superior to others at 20°C but there was no significant difference among fungicides at all other temperatures.

BUCHHOLTZ (6) designed an experiment of 36 pots of naturally infested field soils, planted with 5 beet seed balls per pot, and incubated them at eight different temperatures (5, 9, 12, 15, 20, 25, 30 and 40°C) in 4 replications with uniform soil moisture. No light was allowed to seedlings after emergence. <u>Pythium</u> was recorded from diseased seedlings at all temperatures but in general temperature of 15°C or below was more unfavourable to damping - off organisms than to germination and emergence of seed itself. At 25 to 30°C damping - off was maximum due to activeness of pathogens. BUTLER (7) confirmed the above findings from greenhouse and field experiments at Wisconsin.

From steam disinfected soil, infested with specific pathogens of sugar beets POUND (35) in 1953, followed by LEACH (30) in 1957, found that most severe pre - emergence damping - off in Pythium infested soil occurred at temperatures from 12 -- 20°C, in Rhizoctonia infested soil at 16 - 30°C, in Phoma infested soil at 4 - 20°C, while in Aphanomyces infested soil at 18 - 32°C.

In general these reports on temperature are so variable that no definite conclusion can be drawn from them. Different organisms and hosts have different responses to temperature's effect. There is an indirect relation between temperature and the effectiveness of any chemical seed treatment, and this effect seemed to be a function of the relation of temperature to the proper growth of the treated plants rather than any direct influence of temperature on the chemical used. Mostly the best results of seed treatment on any particular crop are secured near the optimum temperature for the growth of the crop.

b. MOISTURE :

KADOW and ANDERSON (27) studied the soil moisture effect on the incidence of damping - off and its control by excess seed treatments with Semesan 3%, Vasco 4 (Zinc compound) and Cuprocide, at three different moisture levels: lowest, at which merely seed can germinate and grow, medium level and high level (90% moisture to saturation) in unsterilized black silt loam in greenhouse at a soil temperature of 70 to 80^{o}F. Medium to high moisture levels favoured pre - emergence damping - off in lettuce, tomato and beet seedlings. There was no indication that post - emergence phase of damping - off had any direct relation to soil moisture, provided enough moisture is present to allow normal growth of plants. In a similar study of soil moisture effect under greenhouse and field conditions JACKS (23) also reported that pre - emergence damping - off in vegetable crops always occurred at all levels of soil moisture but it was least at medium moisture level (45% moisture holding capacity).

LEACH and HOUSTON (31) observed the influence of moisture on the efficiency and safety of sugar beet seed treatments. They report that application of organic mercuric compounds considerably in excess produced no injury when seeds were planted under ideal soil conditions. Low moisture content of soil exerts little influence on the efficiency of protectants. Storage of water soaked Ceresan treated seeds in airtight container for 1 - 2 days produced significant injury to seeds. Addition of 10% moisture to treated seeds and storage for 10 days before planting retarded germination and produced stunted seedlings, where as 2% added moisture was non-injurious.

Though the influence of soil moisture on damping - off can not be separated from other environmental factors but on the whole damping - off is favoured by wet damped soil conditions.

c. SOIL TYPE AND SOIL REACTION :

In relation to soil reaction and soil type BUTLER (7) recorded in Switzerland that Pythium, Phoma and Rhizoctonia can attack young seedlings of sugar beets only when soil is alkaline. He further indicated that soil deficite in organic matter is usually more heavily contaminated with these fungi than rich soil of loose friable texture. MONTEITH (34) found that Rhizoctonia spp. would grow over a long range of soil p.H. (2.4 to 10.4), with an optimum slightly acid or near the neutral point, BUCHHOLTZ (4) reported that damping - off of alfalfa, caused by Pythium spp. was most severe on acid soils.

On the contrary KADOW et al (27) reported that the soil type in Illinois State is not an important variable in connection with damping - off diseases of vegetable crops. They further summarized from subsequent studies of soil reaction that soil acidity seems not to be a very important factor in the incidence of damping - off of vegetable crops, in so far as Pythium and Rhizoctonia spp. are concerned.

From these variable results reported no definite conclusion can be made about the soil type and soil reaction in relation to damping - off severity, but as a rule p.H. at both extremes interferes with the effect of chemical seed treatments.

d. CULTURAL PRACTICES :

COONS et al in greenhouse tests found that growing sweet clover or alfalfa for 20 to 30 days greatly increased the damping - off in sugar beet seedlings that followed these legumes. Corn grown for the same period significantly lessened damping - off in subsequent sugar beet plantings. Beans as a preceding crop to sugar beets did not apparently alter the initial disease incidence. Field evidences have been obtained in cases where sugar beets immediately followed sweet clover or alfalfa and got poor stands of sugar beet plantings. In contrast beneficial results were obtained when sugar beets followed corn (9).

BUCHHOLTZ (4,5) observed that Pythiaceous fungi parasitize alfalfa seedlings abundantly in the upper 3 to 6 inches of top soil of Webster silt loam and clay loam soils. In an area of Webster silt loam that had been fallowed for two years, the emergence of alfalfa seedlings was 39%, while on an adjacent area which had grown a crop of corn the year before and had been spring ploughed, the emergence and stand was 75%. When the top two and the next two inches of soil were interchanged the Webster silt loam soil produced 80% stand of alfalfa. April or early May field plantings of alfalfa and other legumes and sugar beets in Southern, Central and North Iowa (U.S.A.) yielded uniformly good emergence and also the survival of healthy stands were much more superior to those from late May plantings.

LEUKEL (33) reported that sugar beets planted immediately after spring ploughed sod of legumes had been subjected to a peak population of damping - off pathogens.

From greenhouse age resistance studies with seedlings of alfalfa and other forage legumes which were inoculated at 2, 4, 6 & 8 weeks ages with pure culture of Pythium spp. grown in a white silican sand at 68 - 75°F and watered with modified Hoagland solution, HALPIN (18) examined seedlings 15 days after inoculation and found that none of them had caused any root discoloration on plants. So he concluded that after 2 weeks age there was no attack of post - emergence damping - off due to Pythium spp.

e. CONTROL OF DAMPING = OFF:

ALFALFA:

Since 1934 some information has been accumulated concerning the response of small seeded legumes to seed treatment chemicals, performed in the greenhouse and/or in spaced rows planted in the field. HORSFALL (22) adequately reviewed the literature through 1934. The trend of the results obtained in greenhouse and field row tests seemed to indicate some beneficial results from treatment of forage legume seeds with fungicides but it is important to note that neither the greenhouse nor the field test results had consistantly favoured the practice of treating forage seeds.

BUCHHOLTZ (3) obtained increased stands of alfalfa, sweet clover and Lespedeza by treating the seeds with organic mercury compound dust before planting in Pythium infested soil. Also in field conditions seedlings stand was improved from 8 to 14%, depending upon the soil infestation. Highly significant increases in stands of these crops were obtained by CHILTON (8) with certain fungicidal seed treatments for certain species of these crops. Improved Ceresan proved to be the best seed protectant for the control of post - emergence damping - off of these crops.

ALLISON et al (1) got no significant difference in stands of six forage legumes treated with 5% Spergon, Improved Ceresan and others from field tests but highly significant increases in stands were secured when treated seeds were planted in compost infested with damping - off fungi. VLITOS et al (38) performed seed treatment tests of some legumes including alfalfa with Spergon, Phygon, Arasan, and Ceresan M which was followed by inoculation with Nitrogin bacterial inoculum. Alfalfa treated with Phygon 1%, 0.5% gave a highly significant increase in emergence as compared with check untreated. Ceresan M proved to be injurious to all legumes. Nodulation was not inhibited by seed treatment if it is followed by inoculation.

KREITLOW et al (29) report the results of greenhouse experiments that in absence of Pythium emergence of alfalfa and red clover was not affected by treating seeds with New improved Ceresan, Spergon and Arasan but all protected seedlings from damping - off in Pythium infested soils. In field tests red clover responded better to seed treatment than alfalfa. GERDEMANN (14) reports that seed treatment with Ceresan and Phygon reduced the stands of alfalfa, red clover and sweet clover in dry field soils.

According to KERNKAMP (28) increased seedling stands of alfalfa, red clover and sweet clover were obtained in the greenhouse by seed treatment but in field row tests only alfalfa was consistantly benefited from seed treatment with Spergon and Arasan at a dose of 8 ozs./100 lbs seeds each. It was emphasized that if alfalfa seed had been mechanically injured or if it was of lowered quality for any reason it would respond to seed treatments. HANSON (20) reports no significant increases in stands of these crops from seed treatments with Arasan and Phygon when seeds were planted in silt loam soils of Wisconsin but found significant increases in stands from plantings of muck soil, which is least good for forage legumes production.

In 1952 WEBER (39) found Phygon, Arasan and DOW 9B ineffective for the control of post - emergence damping - off caused by Colletotrichum trifolii Bain. in alfalfa but all were effective to improve total emergence from pre - emergence damping - off. FULKERSON and LEUKEL, in 1953 both worked separately and secured similar results of increased stands in some species of small seeded legumes from seed treatment experiments but some spp. were injured by certain fungicides when planted in dry soils (13, 33). Captan treated seed gave significantly higher emergence of alfalfa seedlings but post - emergence damping - off was not prevented by it. (2, 25). WELTZIEN (40) obtained a significantly increased stand of alfalfa seedlings from seed treatments with Orthocide and C.O.B.H. in greenhouse, especially when the soil was infested with Pythium spp. No damping - off was observed in field experiment.

TYLER et al (37) performed seed treatment field experiments in New York and countryside with forage legumes treated with Arasan and Phygon at a dose of 6-8 ozs./100 lbs. seed, sown in May and early June. Seed treatment neither increased nor depressed the stands and yield of forages. The following year JOHNSON (26) confirmed their findings from field tests at Mississippi. During the same year ATHOW (2) found that seed treatment with organic fungicides increased stands of alfalfa up to 14% while that of clover was increased up to 5% under field conditions.

SUGAR BEETS:

LEACH et al (31) obtained highly significant increases in stand of sugar beet seedlings from seed treatments with Cuprous oxide especially when damping - off was due to Pythium spp. JACKS (24) tried mixed protectants on vegetable seeds sown both in greenhouse and in field and found no advantage gained - over their separate use as seed treatment. Cuprous oxide improved the emergence of beet seedlings but all concentrations of Spergon depressed the emergences.

In greenhouse experiments LEUKEL (33) controlled both pre and post - emergence damping - off in sugar beets by Arasan 4 lb/ acre combined with phosphatic fertilizer and mixed in soil before planting. However under field conditions this treatment was not effective. Seed treatments with Ceresan M, Arasan and Phygon controlled effectively the total damping - off. Recently in 1959 DAVIS et al (11) found Captan and Tennam (manganous dithiocarbamate) the most effective for the control of damping - off of sugar beets in infested steamed soil and unsteamed muck soil.

These reports illustrate that seed treatment of alfalfa and sugar beets controlled damping - off in many cases. The beneficial effect depends, however largely on the species of pathogen present, the variety of soil and the various environmental conditions. The possibilities for damping - off control by seed treatment, therefore, has to be studied under different agricultural conditions before it can be recommended to the growers as a routine method. Studies therefore, were conducted under greenhouse and field conditions.

MATERIALS AND METHODS

Greenhouse Experiments with Treated Seeds :

Crops used in seed treatment experiments were alfalfa and sugar beet. Seed of both crops was obtained from the A.U.B. Agricultural Research and Educational Centre (AREC) in the Beka'a, Lebanon. Alfalfa was an improved A.U.B. Synthetic - 2^{+} while sugar beet was Pedigree - E.$^{\#}$

Viability of seed for both the crops was studied by germination tests of untreated seed. The reported germination percentages are the averages of four replications of 100 seeds of alfalfa and 100 seed balls of sugar beet. Seeds were placed in petri dishes on moist filter paper and incubated at room temperature about (21°C) for a week. In the same way germination tests were also run for treated seeds to find out the effect of fungicides on the germinability of the seed.

The soil used was a black loam to which 20 percent sand was added. This soil had been used many years for the culture of various crops and was heavily infested with damping - off organisms. The soil p.H. was 8, measured by glass electrodes. Plantings were watered daily in an effort to maintain optimum conditions for the development of damping - off, and accordingly soil moisture was always medium to high in every test. The air temperature of the glasshouse varied between

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+ Alfalfa syn - 2 is the F2 generation of syn - 1, evolved at A.U.B. (AREC) Beka'a, Lebanon.

Pedigree - E is an imported variety of sugar beet from Germany.

20 and 30^{o}C for all series of experiments. Soil temperatures were not recorded.

Treatment of Seeds:

Seeds of the two crops were treated with eight different fungicides: Ceresan 1.5% Hg (Phenyl mercury acetate), Ceredon (chinon - oxim-benzoyl - hydrozon), Ceredon special (Ceredon + phenyl mercury chloride), Orthocide 50% (N - trichloro - methylmercapto - 4 cyclo - hexene - 1, 2 - dicarboxinide), Rhizoctol (methyl arsenic sulphide), Rhizoctol combi (Rhizoctol + Ceredon), Spergon (tetrachloro - p - benzo - quinone) and T.M.T.D (tetramethyl - thiuram - disulphide).

Three dosages of each fungicide were used for either crops. These were 200 mg. per 100 grams seed, 400 mg./ 100 g. seed and excess seed treatment (approximately 800 mg/100 g. seed. For convenience these were indicated as series I, II, & III respectively for alfalfa and series IV and V for sugar beet. For series 1 & IV 200 mg of dry fungicide of each chemical were weighed on an analytical balance and were mixed with 100 g. seed in glass flasks and thoroughly were shaken for 5 minutes. For series II, 400 mg. of fungicide were mixed with 100 g. seed and were shaken too. For series III & V (excess seed treatment) sufficient amounts of fungicides were added to 100 g. seed and shaken well so that the seed could carry all that would adhere to it. Surplus was shaken off. Treated seed was stored in dry storage for 10 days but usually seed was planted 3 - 4 days after treatment. Nontreated control seed was always included in all series as check.

Planting the Seed:

A randomized split plot design with 4 replications was used in all greenhouse series. Wooden flats of 50 x 30 x 20 cm. were filled with screened soil uniformly, levelled and labelled too. Six hundred treated seeds of alfalfa were hand planted at a depth of $\frac{1}{2}$ to 1 cm. in three rows per replication in series I, II & III. For sugar beet 50 treated seed balls per replication were planted at a depth of 1 to $1\frac{1}{2}$ cm. in series IV & V. Checks (untreated seeds) with the same number of replications were simultaneously planted in each flat with every treatment. (Fig. I).

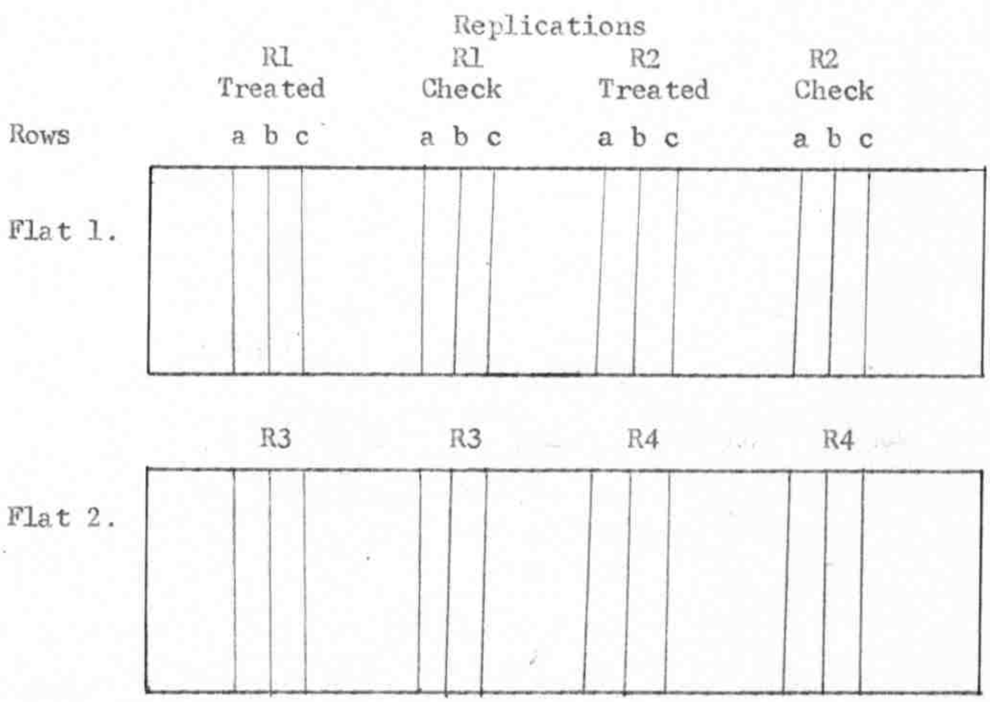


Fig. 1. Layout of planting in flats for greenhouse series.

Obtaining Data:

Data for damping - off were collected 7 to 8 days after planting. In general two phases of damping - off can be recognized: the pre - emer-

Orthocide treated

Check

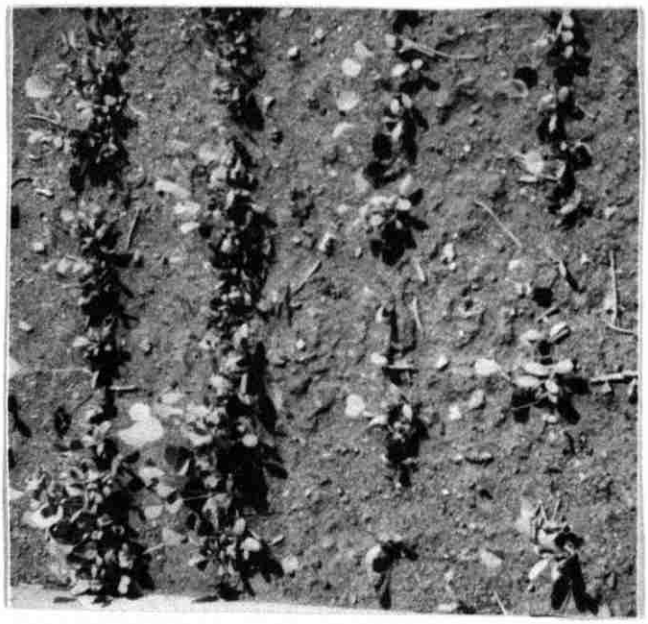


Fig. 2. Pre - emergence damping - off in alfalfa.

gence and the post - emergence damping - off. In the 1st phase the young seedling is killed before it can push through to the soil surface. (Fig 2). Often poor stands of crops both in greenhouses and fields are caused by this phase of damping - off.

The post - emergence phase is characterized by the toppling over of infected seedlings at any time after they emerge from the ground until the stem is hardened and has gained sufficient age resistance. Infections usually occur at or below the ground level and the infected tissues appear soft and water soaked and seedlings look drooping, wilted, dark green, narrow leaved, often falling on the surface of the soil and show root decay. As the disease advances the stem becomes constricted and girdled, till the plant collapses (Fig.3). Having gained a foothold on a single plant, the disease spreads as long as conditions permit.

Separate counts for healthy and diseased seedlings were made. Calculation of the total emergence percentages was based on the emergence of the seedlings out of 100 seeds that were planted. By substraction of emergence percentage from the germination percentage of untreated seed, the pre - emergence damping - off percentages were secured. Damped - off seedlings (Fig 3) were considered as post - emergence damping - off. The summation values of pre - emergence and post - emergence damping - off

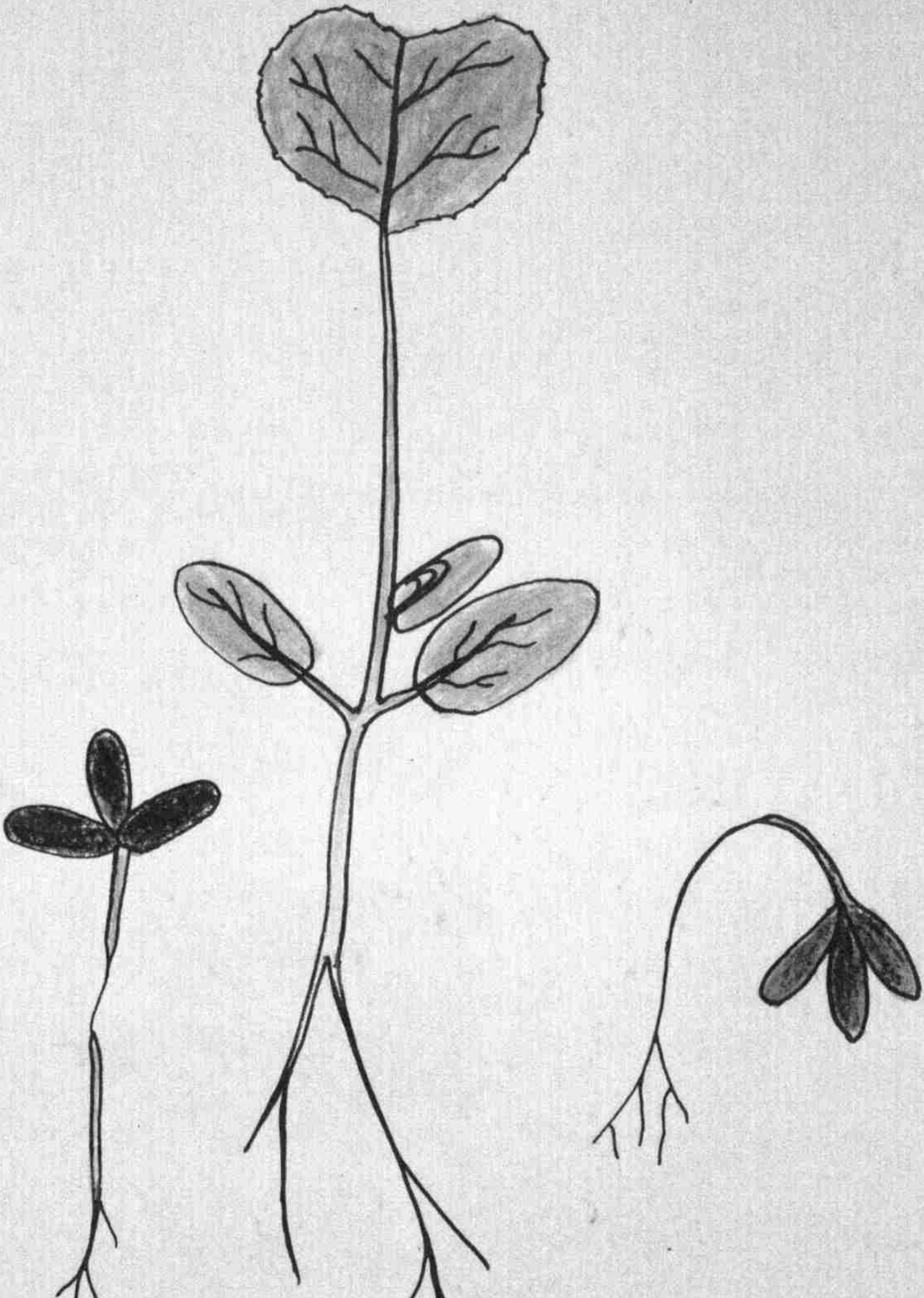


Fig: 3- Post—EMERGENCE DAMPING-OFF
From ten days old plants of alfalfa,
the centre plant is healthy, the
other two diseased.
2 x original size

are the total damping - off. Final healthy stands were obtained when post - emergence damping - off was substracted from emergence percentage.

For statistical analysis the percentage data were changed into transformation values+ and were analysed for pre & post - emergence and total damping - off. Results of each test are listed in separate tables and are the averages, expressed as percentages of all replications.

Field Experiments with Treated Seeds:

Field experiments were conducted for two consecutive years, 1963 and 1964 on the AREC, 80 kilometres East of Beirut under irrigated soil conditions of the Beka'a plain. The soil was a clay type with a p.H of 8.0. This field had grown forage crops of legumes and grasses the year before.

Crops Planted:

Varieties of the two crops of alfalfa and sugar beet were the same as used for the greenhouse series. For seed treatments, chemicals and seed treatment procedure were the same as discussed earlier.

+ Snedecor, G.W. Statistical Methods. Iowa State College. Press Inc. 5th. Ed: 318 -- 19, 1962.

Planting of the Seed:

A separate randomized block design with 4 replications was used for each crop.

For Field Test I

750 seeds of alfalfa, treated with 200 mg/100 g. seed, were hand planted on 23rd April, 1963 at a depth of 2 - 3 cm. in an $1\frac{1}{2}$ metre long single row plot size. Row to row distance was 25 cm. Sprinkler irrigation was supplied immediatly after planting and continued with alternate day intervals till the end of experiment. Weeds that grew were uprooted.

Seedling data were collected about 4 weeks after planting and were analysed statistically for emergence percentages through analysis of variance.

Field Test II:

Planting of 50 seed balls of sugar beet per one metre long row plot size (treated with 200 mg/100 g. seed) followed the alfalfa planting of field test I, in the same field on 12th July, 1963. Planting depth was 3 - 4 cm and row to row distance was 25 cm. The soil was kept moist after planting through sprinkler irrigation. Data were recorded for emergence on August 8, 1963 and were analysed by analysis of variance.

Field Test III:

During 1964 field test III with alfalfa seed, treated in excess was run from April 10 to May 6 on the same field and with the same technique and materials of planting as used for field test I. Data were analysed for emergence percentages by analysis of variance.

Weather data were obtained from the Meteorological Station located at the AREC for a period extending from 2 weeks before to 3 - 4 weeks after plantings.

Soil Treatment Experiment in the Greenhouse:

Greenhouse soil was collected and steamed for 45 minutes without pressure at $100^{\circ}C$ in an electric autoclave. Newly made flats were filled with steamed soil and kept for 48 hours at room temperature before planting. All precautions were taken to avoid recontamination with damping - off organisms.

Planting of untreated seeds of alfalfa was done with the same procedure and method as employed for other greenhouse series. Seedling counts were made as discussed earlier.

Isolation and Pathogenicity Test:

Isolation:

One hundred diseased seedlings of alfalfa were uprooted and washed. Tissues were cut from the advancing margin of decay (discoloring portion) and were washed in sterile water. The cut tissues were plated on potato dextrose agar in petri dishes and were incubated at room temperature about ($21^{\circ}C$) for 4 days.

Colonies of pathogens developed from the cut tissues. Inoculation of potato dextrose agar slants were made from the developing colonies and were incubated at room temperature for their propagation.

Pathogenicity:

Pathogenicity tests were made for both the isolates with an air mycelial growth characteirstic and that with a substrate mycelium. Eight isolates of the former and five of the latter were tested for their ability to cause damping - off in alfalfa. The mycelium grown on potato dextrosc agar slants was removed with little agar and mixed with 10 cc. sterile water in a glass tube. The suspension of each isolate was poured onto steamed soil (steamed for 45 minutes without pressure at 100°C) in three replicates with uniform soil moisture and weight, in glass beakers of 400 cc. capacity. Forty eight hours after inoculation 50 seeds of alfalfa being surface disinfected with 1.5% sodium hypochlorite, were planted in each beaker. Checks of disinfected seeds were also planted in noninoculated steamed soil. Beakers were covered and kept for 7 days at room temperature.

Data were taken for emerged healthy and diseased seedlings 7 days after planting. An analysis of variance was calculated for the data of emergence and total damping - off caused by the isolates.

EXPERIMENTAL RESULTS

Germination Tests with and without Chemical Treatments:

Viability of seed and the phytotoxic effect of chemicals on both the crops were obtained from the germination tests of the treated and untreated seeds, Table 1.

Germination of alfalfa seeds was not affected by chemicals at all dosages. All chemicals except Orthocide and T.M.T.D. were phytotoxic to sugar beet even at the minimum dose of 0.2% and reduced germination percentages significantly below the check. Orthocide and T.M.T.D. both at the 0.2% dosage as well as at excess dosage of seed treatment did not affect the germination significantly.

Greenhouse Series I:

In this series 8 different fungicides were tested on alfalfa seedlings. The results are presented in Table 2. The differences between treatments for emergence are highly significant at 0.01 L.S.D. level. Seed treatments with all fungicides increased emergence significantly above that of the check. Orthocide 50%, Ceredon and Ceredon special as a group produced highly significant increases in emergence above the rest of the chemicals.

Rhizoctol combi, T.M.T.D and Ceresan did not differ significantly for emrgence but constitute a group which is superior to Spergon and Rhizoctol.

TABLE I. Seed germination percentages with and without chemical treatments. Figures are the averages, expressed as percentages, of 4 replications of 100 seeds each.

Treatments	Check seed	Ceresan		Ceredon		Ceredon special		Rhizoctol		Orthocide		Rhizoctol combi		Spergon		T.M.T.D.	
	Germination percentages when seed was treated with:																
Crops	Untreated	d_1	d_3	d_1	d_3	d_1	d_3	d_1	d_3	d_1	d_3	d_1	d_3	d_1	d_3	d_1	d_3
Alfalfa	89.0	89.5	88.0	87.5	88.0	91.0	89.0	88.5	89.5	89.0	88.5	92.2	87.8	85.8	88.7	87.0	89.4
Sugar beet[a]	78.0	45.5	39.3	58.2	40.6	56.0	41.6	59.5	46.0	74.4	70.3	62.0	38.0	60.0	35.2	73.2	65.0

a. Germinated beet seed ball was considered a single seed.

d_1 Denotes the dosage of 200 mg/100 g. seed.

d_3 " " " " excess seed treatment.

TABLE 2. Chemical seed treatments, Greenhouse series I. (200 mg/100 seed):
Effect on Damping - off in Alfalfa.
Figures are the averages, expressed as the percentages of four replications of 600 seeds each.

Treatments	Total Emergence	Pre - Emerg. damping - off c	Post Emerg. damp. - off d	Total damping-off e	Final healthy stand
Check (seed untreated)	9.0	80.0	8.1	88.1	0.9
Ceresan	54.9	34.1	23.3	57.4	31.6
Ceredon	67.0	22.0	24.0	46.0	43.0
Ceredon special	61.9	27.1	23.7	50.8	38.2
Rhizoctol	48.0	41.0	40.4	81.4	7.6
Orthocide 50%	68.9	20.1	34.9	55.0	34.0
Rhizoctol Combi.	58.2	30.8	38.6	69.4	19.6
Spergon	45.7	43.3	42.3	85.6	3.4
T.M.T.D.	57.5	31.5	50.6	82.1	6.9

c. Adjusted to seed germination percentage (alfalfa 89).
d. Damped - off seedling counted & removed
e. Figures are the sum of Pre & Post - Emerg. Damping - off.

The residual effect of chemicals is partly evaluated by post-emergence damping - off. Ceresan, Ceredon and Ceredon special were most effective in preventing the post - emergence damping - off but T.M.T.D, Spergon and Rhizoctol were less efficient in controlling the post - emergence damping - off. Orthocide was less effective in combatting the post - emergence damping - off than it controlled the pre - emergence damping - off.

Consequently the final healthy stands were highest for Ceredon and Ceredon special and smallest for Spergon and T.M.T.D. Orthocide did fair in increasing the stand and ranked next to the Ceredon group. For the comparison of means the reader is referred to table (12, 13).

<u>Series II</u>:

In this test alfalfa seed treatment rate was 400 mg/100 g. seed and was run from March 5 - 13, 1964, under similar conditions as mentioned for series I. Results are given in Table 3. They also indicate that seed treatments increased emergence and stands significantly above the check. Here again Ceredon, Ceredon special and Orthocide gave a higher emergence than the rest of chemicals.

In general the emergence percentages in this series for every chemical has an increasing trend as compared to series I, but they do not differ significantly from series I. Also a trend of controlling the total damping - off is found in this series, indicating that the higher rate of seed treatments can be justified.

Analyses of variances for emergence and total damping - off are given in table (14, 15) in the appendix.

TABLE 3. Chemical seed treatments, Greenhouse series II (400 mgm/100 grm seed): Effect on Damping - off in Alfalfa.
Figures are the averages, expressed as the percentages of four replications of 600 seeds each.

Treatments	Total Emergence	Pre - Emerg. Damping - off c	Post Emerg. Damping - off d	Total damp. - off e	Final healthy stand
Check, seed untreated	14.8	74.2	12.2	86.4	2.6
Ceresan	58.9	30.1	25.8	55.9	33.1
Ceredon	67.6	21.4	25.0	46.4	42.6
Ceredon special	63.4	25.6	25.3	50.9	38.1
Rhizoctol	51.1	37.9	41.4	79.3	9.7
Orthocide 50%	70.2	18.8	34.9	53.7	35.3
Rhizoctol Combination	60.3	28.7	40.2	68.9	20.1
Spergon	54.0	35.0	42.1	77.1	11.9
T.M.T.D.	61.1	27.9	53.1	81.0	8.0

c. Adjusted to seed germination percentage (alfalfa 89)

d. Damped - off seedlings counted & removed.

e. Figures are the sum of Pre & Post - Emergence damping - off.

TABLE 4. Chemical seed treatments, Greenhouse series III <u>excess treatment</u>.
Effect on Damping - off in Alfalfa.
Figures are the averages, expressed as the percentages of four replications of 600 seeds each.

Treatments	Total Emergence	Pre - Emerg. Damping - off c	Post-Emerg. Damping - off d	Total damp. - off e	Final healthy stand
Check, seed untreated	14.4	74.6	13.3	87.9	1.1
Ceresan	54.9	34.1	4.7	38.8	50.2
Ceredon	57.3	31.7	11.1	42.8	46.2
Ceredon special	57.4	31.6	3.7	35.3	53.7
Rhizoctol	54.4	34.6	2.8	37.4	51.6
Orthocide 50%	69.6	19.4	4.4	23.8	65.2
Rhizoctol Combination	52.0	37.0	11.4	48.4	40.6
Spergon	55.7	33.3	9.4	42.7	46.3
T.M.T.D	53.0	36.0	9.4	45.4	43.6

c. Adjusted to seed germination percentage (alfalfa 89)

d. Damped - off seedling counted & removed.

e. Figures are the sum of Pre & Post - Emergence damping - off.

Series III :

Here the seed treatment was done in excess which was found to be approximatly 800 mg/100g. seed. The test was run from November 13-20, 1963.

The results given in table 4 are similar in general to those of series 1 & 2 for total emergence. But the residual effect of chemicals due to excess treatment in this series has consistantly improved the final healthy stands. Orthocide was the best treatment in producing the highest stand of 65% which is significant at 0.01 L.S.D. level.

Ceredon itself could not produce better stand than Ceredon special, Rhizoctol and Ceresan, but was equally efficient as Rhizoctol combination, Spergon and T.M.T.D. Spergon and T.M.T.D hardly gave stands about 8% in series I & II but have increased it by 80% in series III.

Series IV :

In this series sugar beet was used for seed treatment studies at a dose of 200 mg/100 g. seed. All other conditions were maintained like those in previous tests. Planting of beet seed balls was done on April 5 and seedling counts were made on April 14, 1964. The results given in table 5 are variable. Treatments differ significantly for emergence only. Ceresan and Ceredon special did not differ significantly from check but all the rest of chemicals increased the emergence significantly above the check. Rhizoctol combination and Spergon produced the highest emergence which is almost double to that of check.

TABLE 5. Chemical seed treatments, Greenhouse series IV (200 mg/100 g. seed):
Effect on Damping - off in Sugar beets:
Figures are averages, expressed as the percentages of four replications of 50 seed balls each.

Treatments	Total Emergence	Pre - Emerg. Damping - off c	Post - Emerg. Damping - off d	Total damping - off e	Final healthy stand
Check, seed untreated	39.9	38.1	5.6	43.7	34.3
Ceresan	44.0	34.0	2.0	36.0	42.0
Ceredon	66.6	11.4	8.0	19.4	58.6
Ceredon special	44.0	34.0	3.0	37.0	41.0
Rhizoctol	58.5	19.5	16.0	35.5	42.5
Orthocide 50%	60.0	18.0	14.0	32.0	46.0
Rhizoctol combination	69.0	9.0	10.5	19.5	58.5
Spergon	68.0	10.0	11.5	21.5	56.5
T.M.T.D.	55.5	22.5	12.6	35.1	42.9

c. Adjusted to germination percentage of seed ball: (78)

d. Damped - off seedlings were removed and counted.

e. Figures are the sum of Pre & Post - Emergence damping - off.

None of the chemicals was effective in combatting the post-emergence damping - off, hence the differences between the final healthy stands were insignificant from that of check. The comparison of means for the treatments is shown in table (18, 19) in the appendix.

Series V :

This series with the excess seed treatment was run from May 6-14. Results are given in Table 6. Treatments differ significantly both for emergence and stand at 0.01 L.S.D. level (Table 20, 21).

Orthocide, Rhizoctol, T.M.T.D and Ceredon as a group produced significantly increased emergence but Rhizoctol gave the highest emergence. Spergon, Ceredon special and Rhizoctol combination were phytotoxic. Spergon and Ceredon special reduced the emergence significantly below the check.

Nearly all the chemicals were effective in preventing the post-emergence damping - off but Rhizoctol was less effective than all others. Orthocide, Rhizoctol and T.M.T.D all increased the final stands significantly but Orthocide was the best and gave the best stand which is double to that of the check.

Field Experiments:

In relation to field experiments weather data were collected for 1963/64, which are given in Table 7.

Field test I :

Chemical seed treatments with a dose of 200 mg/100 g. seed, were evaluated for the stand of alfalfa at the AREC from April 23 to May 19, 1963

TABLE 6. Chemical seed treatments, Greenhouse series IV: (excess treatment): Effect on Damping - off in Sugar beets: Figures are averages, expressed as the percentages of four replications of 50 seed balls each.

Treatments	Total emergence	Pre - Emerg. Damping - off c	Post - Emerg. Damping - off d	Total damping - off e	Final healthy stand
Check, seed untreated	25.5	52.5	2.0	54.5	23.5
Ceresan	37.0	41.0	2.5	43.5	34.5
Ceredon	41.0	37.0	1.5	38.5	39.5
Ceredon special	19.5	58.5	0	58.5	19.5
Rhizoctol	52.5	25.5	5.5	31.0	47.0
Orthocide 50%	51.5	26.5	0	26.5	51.5
Rhizoctol combination	24.0	54.0	0	54.0	24.0
Spergon	12.0	66.0	0	66.0	12.0
T.M.T.D.	45.0	33.0	0	33.0	45.0

c. Adjusted to germination percentage of seed ball (78)

d. Damped - off seedlings were counted.

e. Figures are the sum of Pre & Post-Emergence damping - off.

TABLE 7. Amount and distribution of rainfall and temperature in the year 1963–64, in Bekaa, Lebanon.

Months	Rainfall in m.m.		Mean air temperature in ^oC		R. Humidity		Bare soil temp. at 5 - 10 cm. depth ^oC	
	1963	1964	1963	1964	1963	1964	1963	1964
January	124.1	77.35	7.1	0.95	77.8	73.7	8.35	5.50
February	70.0	73.50	7.4	3.35	68.0	75.6	9.50	6.44
March	82.4	73.60	6.9	9.05	70.4	71.2	10.30	10.84
April	53.3	73.81	12.0	9.63	70.4	66.6	15.56	14.50
May	11.7	74.52	14.0	12.76	70.0	61.7	19.71	18.76

Data were collected from the Meteorological Station located at A.U.B. (AREC) Bekaa.

The results of this test are given in Table 8 and are the averages of 4 replications, expressed as emergence percentages. As post - emergence damping - off was negligible no data could be acertained.

Although some treatments like Orthocide, Ceredon special and Spergon gave higher emergence above that of check. These differences were all insignificant. T.M.T.D, Ceresan and Rhizoctol group produced less seedlings than the check but here again the differences were not significant.

Field Test II.

In this test sugar beet seed treated with 200 mg/100 g. seed, was planted on 12,July, 1963 with all possible care to maintain the same conditions and methods as mentioned for field test I. Seedling counts were made on August 8, 1963. Results are given in Table 8 along with field test I.

Treatments differ significantly at 0.05 L.S.D level. Rhizoctol, Orthocide and Ceredon special were most effective and produced significantly higher emergence and stands. T.M.T.D and Ceresan however reduced the stands as found in test I. In general the seed treatment practice with sugar beet was more satisfactory under field conditions.

Field Test III:

This comparison was made on the AREC with alfalfa seed treated in excess during spring 1964 from April 10 to May 6. The results given

TABLE 8. Chemical seed treatments, Field test I & II (200 mg/100 g. seed).
Figures are the averages, expressed as emergence percentages of 4 replications of 750 seeds of alfalfa and 75 seed balls of sugar beets per replication.

Crops	Check, seed untreated	Emergence percentages when seed was treated with:							
		Ceresan	Ceredon	Ceredon special	Rhizoctol	Orthocide	Rhizoctol combination	Spergon	T.M.T.D
Alfalfa	47.3	40.5	47.1	48.5	44.8	49.2	40.9	47.9	39.5
Sugar beet	41.5	38.0	44.5	53.0	60.5	57.5	45.0	42.5	36.0

	L.S.D 5%	L.S.D. 1%
Sugar beet	7.521	9.212

TABLE 9. Chemical seed treatments, Field test III. (excess seed treatment), Effect on Damping - off in alfalfa as measured by percentages of seedlings that emerged.

Replication	Seed untreated (Check)	Emergence percentages[a] when seed was treated with:-							
		Ceresan	Ceredon	Ceredon special	Rhizoctol	Orthocide 50%	Rhizotol combination	Spergon	T.M.T.D.
1	46.4	46.1	55.3	49.3	48.1	51.3	45.3	50.7	46.5
2	49.1	50.3	45.7	45.1	47.1	50.8	46.0	54.8	39.9
3	45.6	51.3	48.0	47.3	51.3	55.3	47.2	56.3	46.1
4	50.1	54.7	50.7	53.5	48.8	53.9	43.5	52.3	50.4
Average[x]	47.8	50.6	52.2	48.8	48.8	52.8	45.5	53.5	45.7

L.S.D. 5%
8.236

a. Adjusted to seed germination percentage (89)

x. Means do not differ significantly at 5% level.

in Table 9 are the emergence percentages for 4 replications.

The mean values of all chemicals do not differ significantly from the check. The highest stands were produced by Spergon, Orthocide and Ceredon.

Soil Steaming for Control of Damping - off:

The results given in Table 10 were obtained from alfalfa planted in steamed soil, in the greenhouse. The experiment was conducted from January 19 - 29, 1964. The results indicate that soil steaming prevented damping - off completely as expected and produced about 86% healthy stand. The nonemerged seedlings from steamed soil planting may be due to the weakness of the seedlings themselves.

Orthocide excess seed treatment gave 65% healthy stand which is significantly higher than that of the check which produced 2.5% stand. No diseased plant was found in steamed soil planting while about 5% post - emergence damping - off was recorded in Orthocide excess seed treatment planting. Orthocide seemed to be less effective for the control of pre - emergence damping - off than for post - emergence damping - off. These figures however may be variable with the degree of soil infestation.

Pathogenicity tests:

About 50 isolates were obtained from diseased seedlings of alfalfa. These were grouped according to mycelial growth characteristics. Group A included isolates with an air mycelium growing on the surface of the substrate. Group B was characterized by mycelial

TABLE 10. Effect of soil and seed treatment on damping - off of alfalfa seedlings, expressed as percentages of seed planted.
Figures are the averages, expressed as percentages of 4 replications of 600 seed each

Treatments	Total emergence	Non - emerged seedlings [a]	Post - emergence damping - off	Total Damping - off	Final healthy stand
Check (seed and soil untreated).	12.5	76.5	10.0	86.5	2.5^{xx}
(Seed untreated & steamed soil)	85.6	3.4	-	3.4	85.6^{xx}
(Orthocide excess treated seed & untreated soil).	69.6	19.4	4.4	23.8	65.2^{xx}

L.S.D. 0.01 Level
6.13

a. Adjusted to seed germination percentage (89)

xx. Treatments differ significantly at 1% level.

TABLE 11. Pathogenicity of the isolates on alfalfa seedlings.
Figures are the averages, expressed as percentages of three replications of 50 seeds each.

Treatments		Total emergence	Pre - emergence damping - off a	Post - emergence damping - off	Total Damping - off	Final healthy stand
Check (noninoculated)		83.8	5.2	-	5.2	83.8
Group A	38 a	14.0	75.0	13.3	88.3	0.7
	22 b	15.3	73.7	15.3	89.0	0.0
	36 b	14.6	74.7	14.6	89.0	0.0
	38 b	19.3	69.7	19.3	89.0	0.0
	24 b	25.3	63.7	24.6	88.2	0.8
	11 a	17.3	71.6	16.6	88.2	0.8
	12 b	12.0	77.7	12.0	89.0	0.0
	24 a	24.0	65.0	22.0	87.0	2.0
Group averages		17.72	71.28	17.21	88.49	0.51
Group B	23 a	9.3	79.7	9.3	89.0	0.0
	15 a	32.6	56.4	30.6	87.0	2.0
	15 a_1	32.6	56.4	30.6	87.0	2.0
	16 a	24.0	65.0	24.0	89.0	0.0
	17.a	21.3	67.7	21.3	89.0	0.0
Group averages		23.96	66.04	23.16	88.20	0.80

L.S.D. 5%

1. Total Emergence = 6.401
2. Post - emergence damping - off = 7.375

a. Adjusted to germination percentage i.e. 89

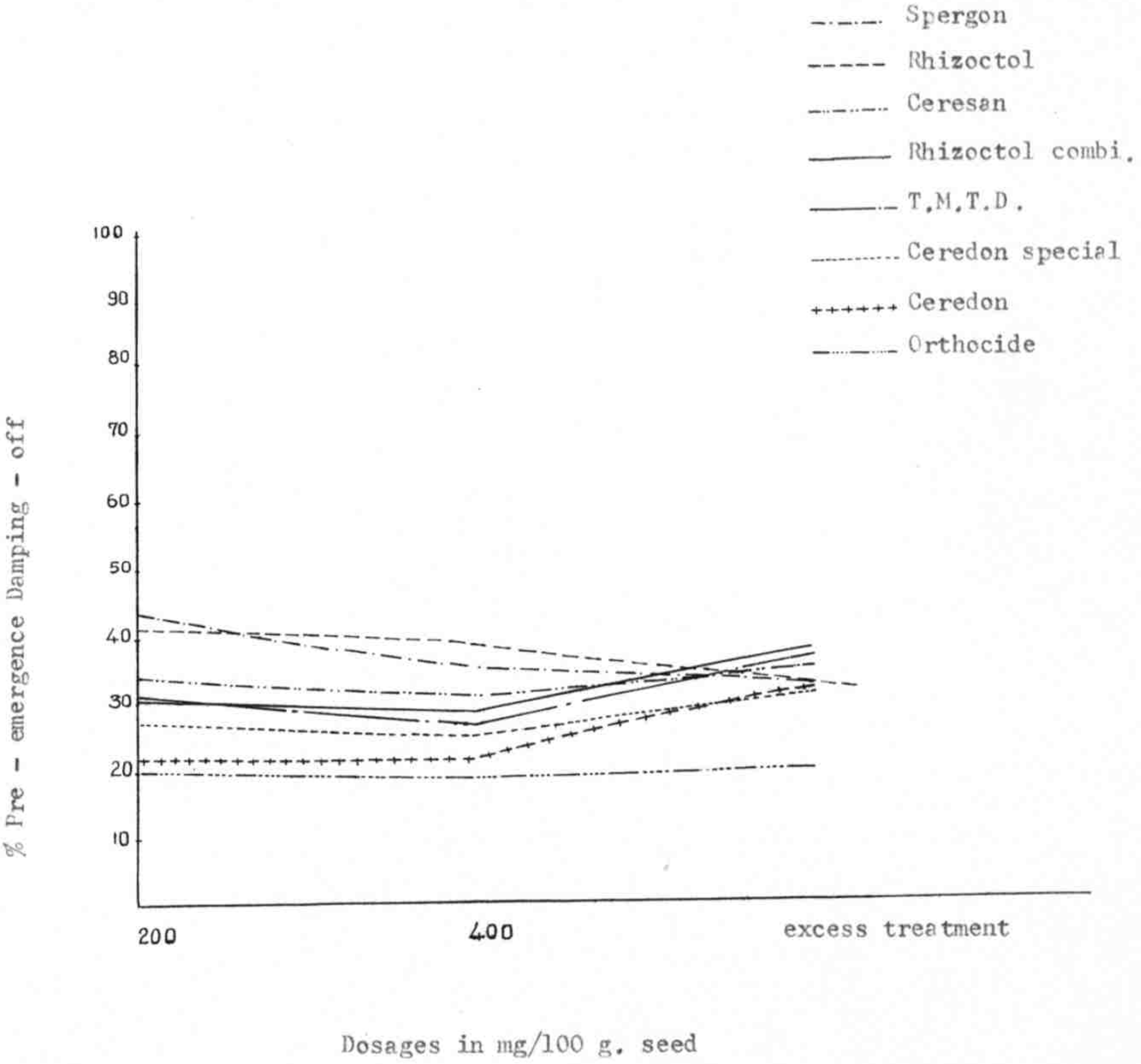
growth inside the substrate.

Thirteen isolates were selected for pathogenicity tests. As table 11 shows all isolates were found to be extremely pathogenic.

Isolates of group A were significantly more pathogenic in causing the pre - emergence damping - off while isolates of group B were more active in initiating the post - emergence damping - off. Isolate No.s 23a, 12b and 38a caused the maximum pre - emergence damping - off.

Noninoculated seedlings remained healthy and produced about 84% healthy stand.

Figure 4. Effect of chemical seed treatments on Pre - emergence damping - off of alfalfa in greenhouse.



DISCUSSION OF THE RESULTS

ALFALFA:

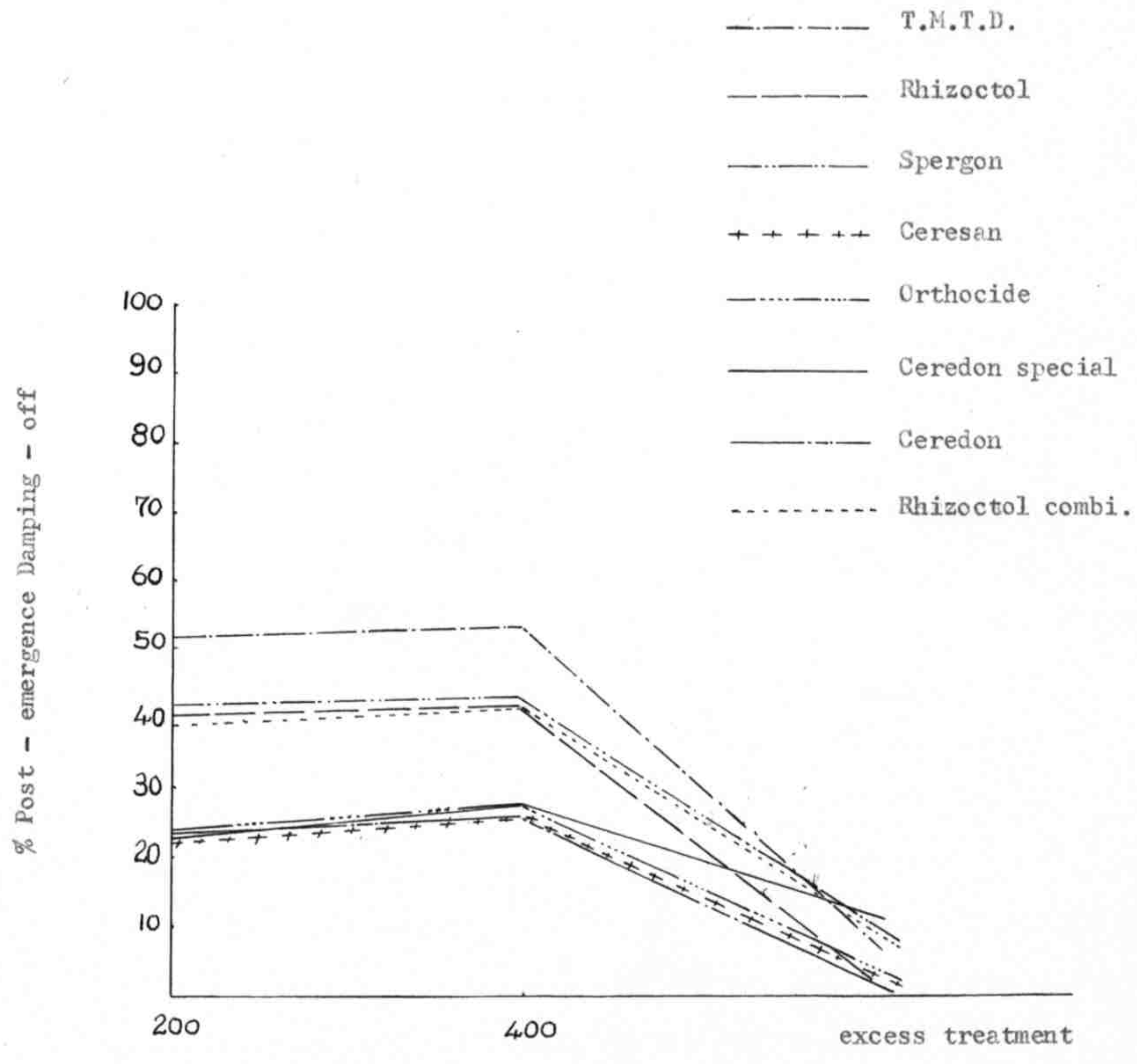
This crop responded most consistantly to seed treatments. The results reported in Tables 2, 3 & 4 are considered extensive enough to warrant some general conclusions and certain specific recommendations.

The results obtained in the greenhouse (Table 2, 3 & 4) were generally in favour of seed treatment. This was specially true when seed was treated in excess and soil was infested with damping - off organisms. As Fig. 4 indicates, little advantage was gained by higher dosages in control of pre - emergence damping - off. Post - emergence and total damping - off (Figs. 5 & 6) however showed a marked decrease with increasing dosages for all chemicals. These results are in agreement with results presented by others, ALLISON (1), BUCHHOLTZ (3), CHILTON (8), KERNKAMP (28), VLITES (38) and WELTZIEN (40).

So far as chemicals are concerned. Orthocide 50% was in most cases superior to all others and can safely be used in excess seed treatment. Ceredon group and Ceresan were nearly as effective in increasing stands as Orthocide. These findings are also in agreement with earlier reports by ATHOW (2), JACKS (25), and WELTZIEN (40).

Under field conditions (Table 8 & 9) although seed treatments gave higher stands than the checks in most cases, all these differences were insignificant. Similar findings are reported by others (1, 14, 20, 29 & 40).

Fig.5. Effect of chemical seed treatments on Post - emergence damping - off of alfalfa in greenhouse.



Dosages in mg/100 g. seed

To explain the failure of improving the stand in the field, one may consider that the soil infestation was low. Also other factors as temperature and soil moisture may have been less favourable for the pathogens in the field. This argument has been proposed by HANSON (20) and KERNKAMP (28).

However seed treatment should be recommended even though the results of field tests were not consistantly favouring it, because alfalfa seed is very expensive while the cost of treating seed or of buying treated seed is negligible. On the other hand the benefit of the treatment may be very high as the greenhouse tests of this study and other reports (1, 3, 8, 28, 29, 38 & 40) have indicated.

SUGAR BEET:

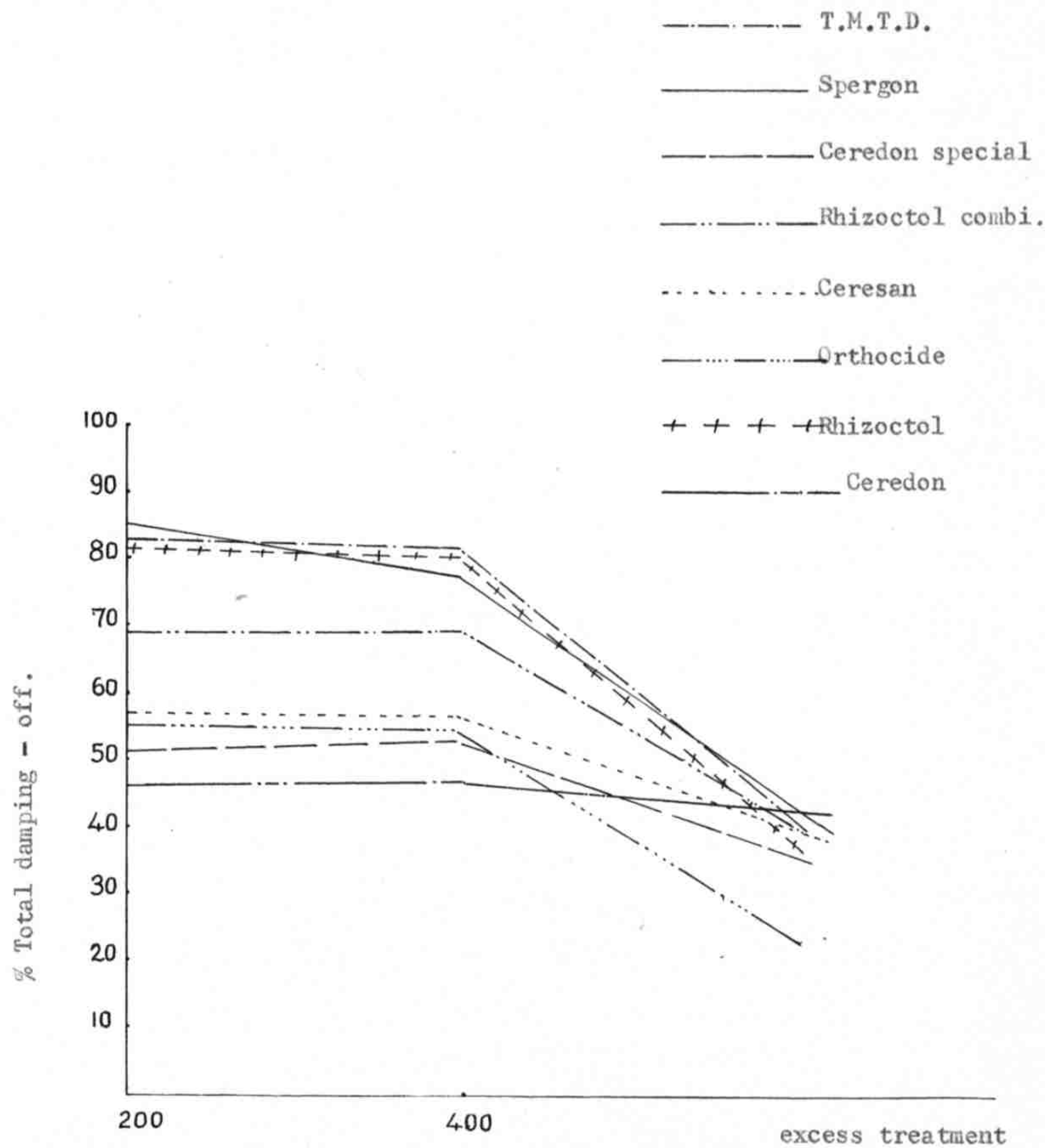
In sugar beet seed treatment results (Table 5 & 6) were variable.

In greenhouse seed treatments with a dose of o.2% (Table 5) have produced significantly higher emergences above the check. Rhizoctol combination and Spergon were the most effective. None of the chemicals could prevent post - emergence damping - off. Therefore the differences between the final stands were insignificant.

Similarly with excess seed treatment most of the chemicals produced higher emergence and stands than the check but Spergon and Ceredon special lowered emergence and stand significantly.

In the phytotoxicity test (Table I) Spergon and Ceredon group significantly impaired the seed germinability. JACKS (24) found similar results with Spergon and Ceredon special. The trend of phytotoxicity

Fig. 6. Effect of chemical seed treatments on total damping - off of alfalfa in greenhouse.



Dosages in mg/100 g. seed

due to higher dosages is revealed from Figs. 5 & 6. In all cases emergence and stand were higher for lower dosages of seed treatment.

Chemicals were mostly found phytotoxic. Nevertheless the concentration of the chemicals is diluted when treated seeds are planted in the soil. Thus they become less phytotoxic. This is specially true when seed treatment rate was at 0.2%. The effect of chemicals then, becomes pronounced in combatting damping - off organisms. LEUKEL (33) and LEACH (31) report similar results.

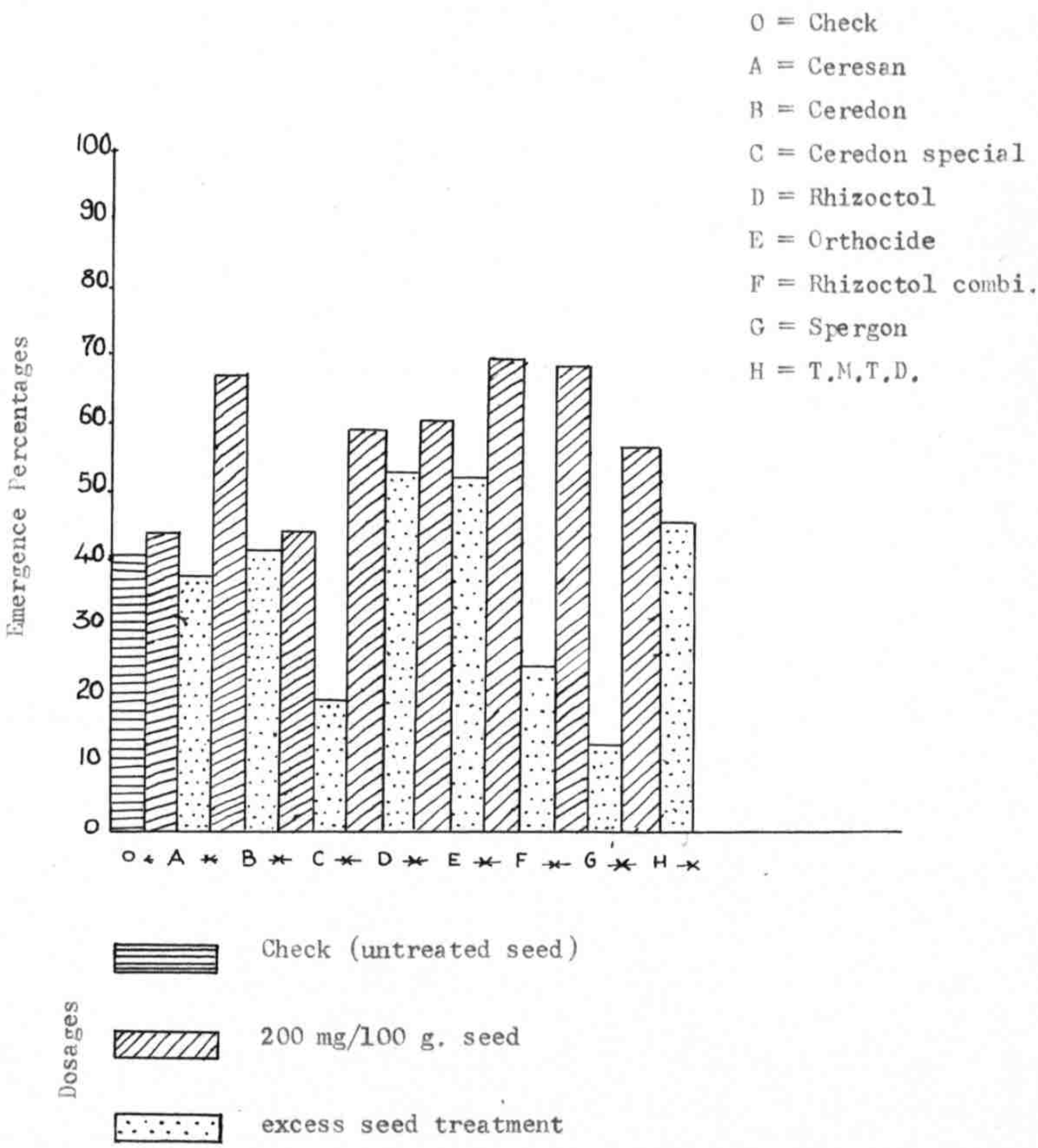
Orthocide and T.M.T.D being ineffective on germinability (Table I.) increased the emergence and stands significantly with all dosages. These results are in support to those reported by DAVIS (11).

From field studies (Table 8), the results with sugar beets are in favour of seed treatment. Orthocide, Rhizoctol and Ceredon group produced significantly higher stands above the check.

As sugar beet planting followed alfalfa immediatly after digging the seedlings, a richer fungus flora was probably created which was combatted effectively by the chemicals. COONS (9) and WILLIUM (41 & 42) report similar results from crop rotation studies.

In general seed treatment practice for sugar beet is worthwhile but it is important to note that the rate of seed treatment should not exceed the recommended dose of 0.2% for mercury compounds. Orthocide and T.M.T.D. both can be used even at higher dosages.

Fig. 7. Effect of chemical seed treatments on emergence of sugar beet seedlings in greenhouse.

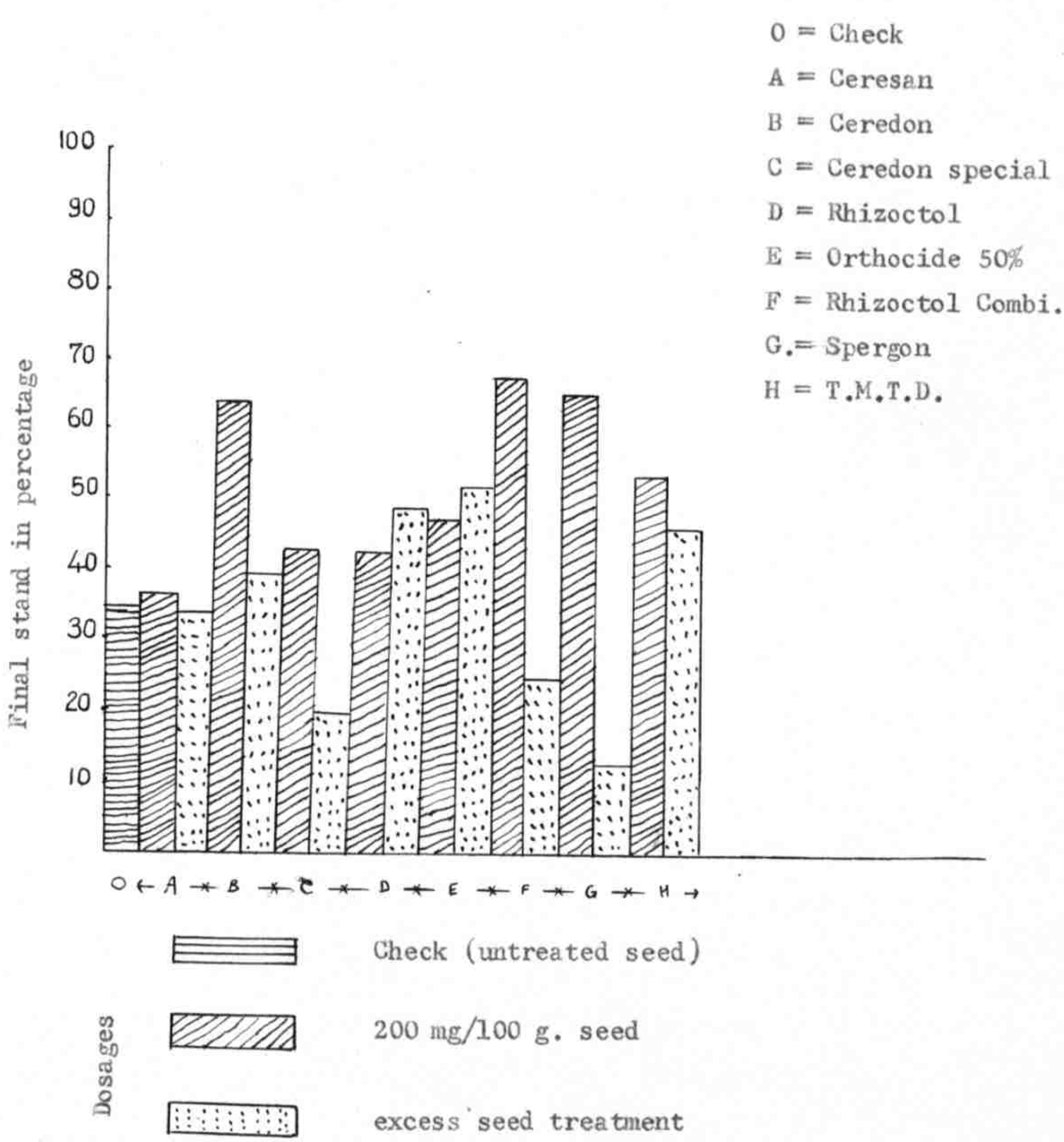


Soil Steaming:

Although soil steaming completely controlled damping - off (Table 10) of alfalfa seedlings, it is imperative that extra precautions must be taken to prevent recontamination, which makes the soil steaming method of limited value for damping - off control in the hands of average growers. When recontaminations occur then due to lack of antigonism the disease is often more serious than it would have been, had the seed been planted in unsteamed soil.

Under field conditions the soil steaming method has no practical value due to its high cost of operation. Also seed-borne damping - off pathogens can easily develop in steamed soil and may cause severe losses of stands. Seed treatment is therefore also recommended, when steamed soil is used for damping - off control.

Fig. 8. Effect of chemical seed treatments on final healthy stand of sugar beet seedlings in greenhouse.



SUMMARY AND CONCLUSION

Some of the recommended methods of control for damping - off of alfalfa and sugar beet were evaluated under greenhouse and field moist soil conditions.

Evaluation of Seed Treatments:

Alfalfa

Eight different fungicides were tested for phytotoxicity and their effectiveness on controlling the seedling diseases:

1. Germination was not affected.
2. All chemicals consistantly increased the stands under greenhouse conditions.
3. Orthocide 50% was most effective, especially when seed treatment was in excess.
4. Ceredon, Ceredon special and Ceresan at excess dosages were nearly as good as Orthocide
5. Under field conditions none of the chemicals increased the stand significantly over the check.

Sugar beet:

1. All chemicals except Orthocide and T.M.T.D, reduced the seed germinability significantly, even with a seed treatment dose of 0.2%.
2. In the greenhouse Spergon and Rhizoctol combination with 0.2% dosages produced significantly higher emergence above all treatments but impaired significantly the emergence and stand when

excess seed treatment was used.

3. Orthocide, Rhizoctol and T.M.T.D at all dosages produced higher stands both under greenhouse and field conditions

II. Soil Steaming:

Steaming the soil gave the most satisfactory results and prevented damping - off of alfalfa seedlings. When soil steaming method is used it is imperative that extra precautions should be taken to prevent recontamination. This fact alone makes soil steaming method of limited value for damping - off control for average growers. Therefore chemical seed treatments with effective chemical like Orthocide at excess rate can substitute the soil steaming.

III. Pathogenicity Tests:

1. All isolates were found extremely pathogenic on alfalfa seedlings.
2. The pathogens were identified as *Pythium spp.*

APPENDIX

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TABLE 12. Greenhouse series $\bar{I}$ (200 mg/100g. seed).
Analysis of variance for emergence percentage of alfalfa seedlings.

Source	D.F.	M.S.
Replications	3	7.745
Treatments	8	2225.38 xx
Error	52	19.550

xx denotes F values significant at the 1% level.

	L.S.D. 5%	L.S.D. 1%
Chemicals against chemicals:	6.322	8.507
Check " " :	4.739	6.377

Treatments:

	Orthocide	Ceredon	Ceredon special	Rhizoctol, combi.	T.M.T.D.	Ceresan	Rhizoctol	Spergon	Check
	(E)	(B)	(C)	(F)	(H)	(A)	(D)	(G)	(O)
Mean x	56.2	55.0	51.9	49.8	49.3	47.9	43.9	42.5	16.8

x Underlined means do not differ significantly at 5% level.

TABLE 13. Greenhouse series I. Analysis of variance for total damping - off of alfalfa seedlings.

Source	D.F	M.S
Replications	3	8.466
Treatments	8	764.782[xx]
Errors.	52	11.301

[xx] denotes F values significant at the 1% level.

	L.S.D. 5%	L.S.D. 1%
Check against chemicals	3.611	4.858
Among chemicals	4.817	6.481

Treatments:

O	G	H	D	F	A	E	C	B
Means[x]								
69.7	68.2	65.0	58.3	56.4	49.2	47.9	46.9	42.8

[x] Underlined means do not differ significantly at 5% level.

TABLE 14. Greenhouse series II. Analysis of variance for emergence percentage of alflafa seedlings.

Source	D.F.	M.S.
Replications	3	5.910
Treatments	8	1709.428 xx
Error	52	6.171

xx denotes F values significant at the 1% level.

	L.S.D 5%	L.S.D 1%
Chemical against chemicals:	3.558	4.787
Check " " :	2.667	3.588

Treatments:

E	B	C	H	F	A	G	D	0
Mean x								
56.92	55.25	52.83	51.42	50.97	50.15	47.3	45.65	22.50

x Underlined means do not differ significantly at 50% level.

TABLE 15. Greenhouse series II. Analysis of variance for total damping - off of alfalfa seedlings.

Source	D.F	M.S
Replications	3	1.12
Treatments	8	693.445[xx]
Errors	52	2.314

[xx] denotes F values significant at the 1% level

	L.S.D. 5%	L.S.D. 1%
Check against chemicals	1.632	2.196
Chemicals against chemicals	2.177	2.929

Treatments:

O	H	D	G	F	A	E	C	B
Means:								
68.4	64.2	63.0	61.4	56.1	48.4	47.1	45.6	43.0

[+] Underlined means do not differ significantly at 5% level.

TABLE 16 Greenhouse series III. Analysis of variance for emergence percentages of alfalfa seedlings.

Source	D.F	M.S
Replications	3	1.973
Treatments	8	77.764[xx]
Error	52	

[xx] denotes F values significant at 1% level.

	L.S.D 5%	L.S.D 1%
Chemicals against chemicals	7.037	8.513
Check " "	5.274	7.098

Treatments:

	E	C	B	G	A	D	H	F	O
Mean[x]	62.38	53.45	53.45	53.38	51.75	51.47	50.6	49.6	23.25

[x] Underlined means do not differ significantly at the 5% level.

TABLE 17. Greenhouse seriesIII(excess treatment) Analysis of variance for total damping - off of alfalfa seedlings.

Source	D.F	M.S
Replications	3	0.762
Treatments	8	1995.601 xx
Error	52	9.191

xx denotes F values significant at the 1% level.

	L.S.D 5%	L.S.D 1%
Check against chemicals	3.254	4.379
Among chemicals	4.341	5.841

Treatments:

	O	F	H	B	G	A	D	C	E
Means x	69.6	44.0	42.3	41.0	40.8	38.5	37.2	36.4	29.0

x Underlined means do not differ significantly at 5% level.

TABLE 18. Greenhouse series IV. (200 mg/100 g. seed) Analysis of variance for emergence percentage of sugar beet seedlings.

Source	D.F	M.S
Replications	3	160.9
Treatments	8	366.3^{xx}
Error	52	65.75

^{xx}denotes F values significant at 1% level.

	L.S.D 5%	L.S.D 1%
Check against chemicals:	8.869	10.019
Chemicals against chemicals:	11.831	16.033

Treatments:

	F	G	B	E	D	H	A	C	check
Mean^{x}	68.7	65.9	56.0	51.2	50.6	46.8	41.6	41.6	38.8

$^{+}$ Underlined means do not differ significantly at 5% level.

TABLE 19. Greenhouse series V. (excess treatment) Analysis of variance for percentage of sugar beet seedlings.

Source	D.F	M.S
Replications	3	19.9
Treatments	8	339.8^{xx}
Error	24	32.06

^{xx} denotes F values significant at the 1% level.

	L.S.D 5%	L.S.D 1%
Check against chemicals:	8.259	11.192

Treatments:

	D	E	H	B	A	O	F	C	G
Mean^{x}	46.5	46.0	42.2	39.7	37.3	30.2	29.2	26.1	20.3

+ Underlined means do not differ significantly at 5 % level.

TABLE 20 Greenhouse series V. (excess treatment). Analysis of variance for total damping - off of sugar beet seedlings.

Source	D.F	M.S
Replications	3	167.447[xx]
Treatments	8	255.128
Error	24	13.94

[xx] denotes the F values significant at 1% level

	L.S.D 5%	L.S.D 1%
Check against chemicals	5.266	7.136

Treatments:

	G	C	O	F	A	B	H	D	E
Means[x]	54.4	49.9	47.6	47.3	41.2	37.9	35.3	35.1	30.9

[+] Underlined means do not differ significantly at 5% level.