BEN-GURION UNIVERSITY OF THE NEGEV
P.O.B. 653, BEER-SHEVA 84105, ISRAEL

אוניברסיטת בן-גוריון בנגב
ת.ד. 653, באר-שבע 84105

PN ABW-125

Our Ref: 86382

23 May 1995

Office of the Science Advisor
AID/CDR Program - Office of Research
Room 609, SA-18
Agency for International Development
Washington, DC 20523-1815
U. S. A.

Re: AID-CDR Project No. C9-114
Grant No. DHR-5544-G-SS-0074-00
Cultivation of Terfezia pfielii - The Kalahari Truffle
Dr. Varda Kagan-Zur / Dr. Nurit Roth-Bejerano

Enclosed please find four (4) copies of the final Annual Scientific Report submitted by Dr. Varda Kagan-Zur for the above-mentioned project.

With thanks for your cooperation.

Yours sincerely,

Moshe Amir
Director
Department of Research Contracts

cc: Science Attache - US Embassy Tel-Aviv (w/encl 1)
AID Representative, USAID Mission - Botswana (w/encl 1)
CDIE, Washington, DC (w/encl 1)
NAS/BOSTID, Washington, DC (w/encl 2)
Dr. V. Kagan-Zur

RESEARCH & DEVELOPMENT AUTHORITY
Department of Research Contracts
3rd floor, Zalman Aranne Library Building, New Campus
Tel: 972-7-472425/6 | Fax: 972-7-472930 | E-Mail: resco@bgumail.bgu.ac.il

הרשות למחקר ולפתוח
המחלקה לחוזי מחקר
בנין הספריה ע"ש זלמן ארן, קומה ג', הקריה החדשה
טל: 07-472425/6 | פקס: 07-472930 | דאר אלקטרוני:

a

C9-114

THE INSTITUTES FOR APPLIED RESEARCH
BEN-GURION UNIVERSITY OF THE NEGEV

BGUN-ARI-27-95
Limited

CULTIVATION OF *TERFEZIA PFEILII*, THE KALAHARI DESERT TRUFFLE

Annual Report
March 1994-March 1995

V. Kagan-Zur*, F. Taylor, D. Thamage****
and N. Roth-Bejerano***

*Institute for Agriculture & Applied Biology, The Institutes for Applied Research
**Veld Products Research, Botswana
***Department of Life Sciences, Ben-Gurion University of the Negev

Submitted to AID-CDR
(File No. 86382101)

Beer-Sheva, Israel April 1995

REC'D IN R&D/R
JUN 06 1995
CC:BOSTIA

EXECUTIVE SUMMARY

This report covers the fourth and last year of the research on *Terfezia pfeilii*—the Kalahari desert truffle—in the framework of the project funded by the AID-CDR.

Terfezia pfeilii is one of the few perennial food plants in the Kalahari Desert which have the potential to provide food security and to generate significant cash income. The purpose of this research was to study the ecology and biology of the truffle in an effort to develop methods for its agricultural cultivation. A successful conclusion to the domestication of *Terfezia pfeilii* is becoming more important as the options for food security amongst the rural poor and marginalised become fewer and fewer. Botswana government agricultural officers are openly acknowledging that arable agriculture in semi-arid Botswana is not viable no matter how many millions of dollars are poured in to promote "better programmes". The semi-arid climate, erratic rainfall and marginal soils mitigate against arable agriculture. One possible answer lies in developing an unconventional agriculture hinging on indigenous perennial food plants, which are, by definition, totally adapted to the semi-arid ecosystems.

During the 4-year research period, we organized three expeditions into the Kalahari to study *Terfezia pfeilii* Henn. Members of both the Israeli and the Botswana teams participated in the first two, only members of the Botswana team took part in the third. Ascocarps (fruiting bodies) of the truffle were collected during the first two trips, but no truffles were found on the third. Truffles were discovered attached to a 'stalk' consisting of sand particles and a mesh of mycelial tissue. Rhizomorphs were found in the vicinity of this 'stalk'. The ascocarps generally occurred on the southern (shady) side of shrubs and trees, only a few of which had roots that appeared to be slightly mycorrhized. For the most part, plants found to be mycorrhized in such locations were herbaceous monocotyledons or dicotyledonous shrubs and trees.

Soils at *T. pfeilii* sites had a lower than neutral pH and a low calcium content.

Experiments conducted in Israel with the aim of effecting mycorrhization by *Terfezia pfeilii* of potential plant symbionts such as Acacia species, *Grewia flava* or *Cynodon dactylon* roots failed to yield results as clear-cut as experiments using *Terfezia leonis* (an Israeli desert truffle), *Terfezia boudieri* or *Tuber melanosporum* and their respective symbionts. Attempts to isolate putative *T. pfeilii* mycelium from fruiting bodies failed. However, putative mycelia were isolated in Israel from germinated spores early during the first year; although this mycelium shows all the characteristics of a Terfezia mycelium, no *bona fide* mycorrhization was ever obtained (see Research Accomplishments section). Isolation of mycelium from truffle 'stalks' in Botswana seemed at first to be successful, but later the material turned out to be a contamination. *Grewia flava*, *Acacia hebeclada* and *Acacia melifera* plants were inoculated with spores experimentally; some developed mycorrhization, as did *Cynodon dactylon* seedlings; however, a. the ratio of mycorrhized to non mycorrhized plants was consistently low, and the former showed a low degree of mycorrhization, and b. non inoculated control plants also showed occasional signs of mycorrhization, so that conclusions could not be drawn with any certainty.

Results of small scale experiments aimed at mimicking bush conditions in Botswana, were to say the least ambiguous, as plants in control plots were also mycorrhized, and similar local mycorrhizal fungi—probably not *T. pfeilii*—may have mycorrhized the plants in the inoculated plots.

C

A. RESEARCH OBJECTIVES: Our overall aims were twofold: to restore natural production; and to develop means that will enable the rural poor to cultivate truffles for food security and as an indigenous cash crop requiring very little water and cultivable on Kalahari soils using relatively simple agricultural techniques.

Our first specific objective was to study the *Terfezia pfeilii* biology and life cycle, in an attempt to identify with certainty at least one definite plant symbiont (see details below). Some efforts were dedicated to understanding environmental (ecological, pedological) conditions of typical truffle-producing areas.

B. RESEARCH ACCOMPLISHMENTS: For details please turn to the next page.

C. SCIENTIFIC IMPACT OF COLLABORATION: During the research period both groups took part in expeditions into the desert, in pursuit of truffles, their ecology and their biology. Mr. Douglas Thamage trained in Israel and undertook research in Botswana. Information was flowing constantly between partners.

D. DESCRIPTION OF PROJECT IMPACT: No practical results can be reported. One paper, which acknowledges the AID-CDR grant, is about to be published in Mycol. Res.1995

E. STRENGTHENING OF DEVELOPING COUNTRY INSTITUTIONS: 1. Mr Douglas Thamage, who trained in Israel within the framework of this project, undertook research in Botswana using skills obtained in Israel. 2. Purchase of some minor equipment and materials using funds made available by this project, along with the utilization of University facilities, represented a strengthening of research capabilities of Veld Products Research.

F. FUTURE WORK: The *Terfezia pfeilii* biocharacteristics are apparently atypical of other *Terfezia* species. Further research is needed, such as collection of terfezia from other parts of the country where these truffles are reported to occur, to enable comparison of site characteristics (plant habitats, soil composition and climate conditions) with characteristics already known and formulation of general conclusions about this truffle. The possibility of a non-mycorrhizal habit, pointed to our results, should be explored, and introduction of a better understood terfezia symbiont species such as *T. leonis* with either *Helianthemum sessiliflorum* or *Cistus incanus* should be undertaken.

We are planning to continue the research after seeking funds from other sources.

B. RESEARCH ACCOMPLISHMENTS

INTRODUCTION

In our first expedition to the Kalahari desert (Kagan-Zur et al. 1992), we observed that most truffles were found not too far from roots of certain bushes and trees, some of which seemed to be mycorrhized. We concentrated our efforts on the latter, since all truffles known so far, including species of *Terfezia,* enter into symbiosis with such dicotyledonous plants (e.g. Signorini & Valli, 1990; Giovannetti et al., 1994). For almost two years we tried to mycorrhize three species of these plants: two species of *Acacia* (*hebeclada and melifera*) and *Grewia flava*.. Our efforts were totally fruitless in Botswana and only very partly and very seldom successful in Israel (Kagan-Zur et al. 1992, 1993). We therefore undertook a second trip to truffling sites in the Kalahari to further clarify our early findings, as well as to collect some more local information.

On the second trip we noticed that truffles were found mainly on the southern, shady side of shrubs and trees. We were unable to establish definitely that any of these plants were truly mycorrhized, and our above-mentioned limited success in obtaining mycorrhization with some of these plants led us to doubt their singular role as symbionts of *T. pfeilii.* Perennial gramineae plants were present at all the truffle sites. Of all the plant samples taken from the vicinity of the ascocarps and examined under the microscope, only *Cynodon* and *Stipagrostis uniplumis* (L.) de Winter seemed to be well mycorrhized. It seemed therefore worthwhile to examine whether the gramineae functioned as *T. pfeilii* symbionts. Consequently, all our experiments in Israel, and some in Botswana, were focussed on effecting mycorrhization between gramineae and *T. pfeilii.* Results presented last year seemed to favor this possibility, but as controls were also sometimes mycorrhized, during our last research year we concentrated on defining the experimental conditions more sharply.

EXPERIMENTS CONDUCTED IN ISRAEL

1. Effect of low-pH irrigation and addition of a soil bacterium on *C. dactylon* mycorrhization. Last year's low-pH irrigation experiment was repeated, the plants being watered with either dilute HCl or H_2SO_4 solutions (the latter was chosen eventually as being less harmful than chlorides to plants) - adjusted to pH 2.7-3.0 -instead of tap water. A 75% sand, 25% peat and 5% vermiculite soil mixture was used (no. 3) (Kagan-Zur et al. 1994). *Pseudomonas* was again added because last year's results (Kagan-Zur et al. 1994) seemed to indicate enhancement of mycorrhization levels. The pots were kept under a 30% shade net.

While control pots exhibited better survival this year than the year before, spore-inoculated seedlings fared still better, repeating last year's observation (Kagan-Zur et al. 1994). We note that enhancement of seedling survival by *T. pfeilii* inoculation seems to be a general phenomenon, although not always accompanied by observable mycorrhization (Kagan-Zur et al. 1993, 1994). The nature of this phenomenon is not clear at present. It could stem from supply of a nutritional component missing in the soil mixture. Addition of *Pseudomonas fluorescens* culture enhanced the survival, development, and, to some extent, mycorrhization (pots irrigated with HCl) of the inoculated seedlings, the latter finding according with a work claiming that the bacterium acts as a strong antagonist to soil fungi but has less effect on fungi in mycorrhizal association (Mamoun & Olivier 1992).

In this experiment, staining was carried out by the new bleaching-staining method (Koske & Gemma 1989). At the first check, endomycorrhiza was observed in 100% of pots containing *C. dactylon* and inoculated either with spores alone or with spores plus *P. fluorescens* (Table 1). At a later check (of different pots, as sampling destroyed the plants), *Pseudomonas* seemed to enhance association of *T. pfeilii* and *C. dactylon* roots in HCl watered pots (Table 1). Lower levels of association were observed at this later check. Endomycorrhizae do not create uniformly-mycorrhized roots, and colonization depends on root age. Also, every mycorrhized cell has a limited mycorrhization period, after which the endophyte disintegrates and the cell appears non-mycorrhized (Alexander et al. 1988). Such older roots may seem to be ectomycorrhized poorly or not at all mycorrhized. Deducing from the results of our two checks that observation had been initiated too late, we decided that henceforth we would check mycorrhization levels at different times following inoculation (starting three weeks after inoculation when mycelium was used as inoculant and at about three months when using spores). On both checks documented in Table 1, control pots were also found to be mycorrhized, indicating a possible mycorrhitic contaminant.

The reasons for the low incidence of mycorrhization are not understood at the moment. A possible explanation could be that conditions in our experiments were not ideal. For example, low-pH irrigation is not only not sufficient for lowering soil pH (Kagan-Zur et al. 1994), it may by itself cause damage by being momentarily too harsh on roots or fungi, or by adding to soil salinity; soil temperatures in pots may be too high for growing the seedlings (as opposed to plots), light intensity at 30% shade may be too low, etc. But our findings could also mean that *T. pfeilii* is a non-mycorrhizal fungus and that observed mycorrhizae are due to contaminating fungi.

2. Endomycorrhiza versus ectomycorrhiza. We were not sure whether *T. pfeilii* is endomycorrhitic like *T. boudieri* (Awameh, 1981), ectomycorrhitic like *T. leonis* (our

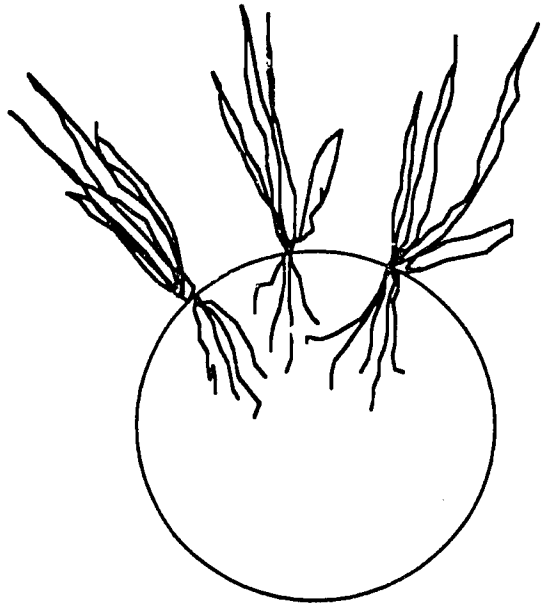
own unpublished results) and *T. leptoderma* (Dexhimer et al. 1985), or capable of developing either form of association depending on nutritional conditions, like *T. arenaria* and *T. claveryi* (Fortas & Chevalier 1992). In some instances we observed endomycorrhizal forms and in others only ectomycorrhizal forms. In a comparative study of the effect of sand or agar support media (pH 5.8) on the inoculation of cynodon or acacia or both with spores under completely sterile conditions *in vitro* (Table 2A), we observed a striking difference between types of mycorrhiza in sand (endomycorrhiza) versus agar (ectomycorrhiza). The ectomycorrhitic nature of the association was not affected by the level of phosphate supply, as lowering the phosphate to 20% of its level in a balanced nutrient solution (Hoagland & Arnon, 1950) did not alter the character of the mycorrhizae, contrary to results reported by Fortas & Chevalier (1992) for*T. arenaria* and *T. claveryi* . Instead, it appears that the type of (supposedly) inert support was the source of the difference.

3. Use of putative *T. pfeilii* mycelium as inoculum *in vitro*. As spore solutions may contain contaminants which may or may not turn out to be mycorrhitic, our isolated putative *T. pfeilii* mycelium was used in further experiments aimed at studying the type of mycorrhiza formed by *T. pfeilii* under different conditions. Results (Table 3A) showed null association in agar media, although hyphal growth was observed in the root vicinity. Either endo- or ectomycorrhizae developed in soil, presumably depending on nutrient supply. Our results could indicate either that agar is not an adequate support for obtaining *T. pfeilii* mycorrhizae, or that the sand contained contaminating mycorrhizal fungi which were not eliminated by a single sterilization cycle.

A different support medium, sterile perlite, was employed to test the first possibility; several different nutrient solutions were tried and the putative mycelium served as inoculant. Only one cynodon seedling was found to be mildly mycorrhized (Table 3B), while hyphae were observable in several, mostly in early checks.

Two sterilization cycles of a sand-containing medium still gave rise to mycorrhizae in some tubes; however, both control and inoculated plants were affected, indicating contamination (Table 3C).

Since the rate of mycorrhization was generally low, we tried a different method of inoculation with mycelium: instead of adding a piece of mycelium containing agar to a test tube containing a suitable seed or seedling—conceivably too low an inoculum density—we added two-week-old seedlings to a petri dish of freshly growing mycelium, as shown below.



Application of this method to *T. leonis* mycelium plus either *Helianthemum sessiliflorum* or *Cistus incanus* resulted in rapid formation of association. However, no association developed when we tried putative *T.pfeilii* mycelium with either *Cynodon dactylon* or *Acacia melifera* , and the hyphae seemed to be recoiling from the roots. Taken together with the results presented in Table 3B, this may mean that cynodon and acacia are not suitable symbionts, or alternatively that the isolated mycelium is not *T. pfeilii*. On the other hand it may also turn out, as was already stipulated earlier, that *T. pfeilii* is not a mycorrhizal fungus.

4. Spore germination capacity. No hyphae were observed 3 and 4 months after inoculation with spores on a medium subjected to four sterilization cycles. This could indicate any combination of the following: a. formation of inhibitor(s) in the medium, as observed for sun-sterilized plots (Y. Kapulnik, personal communication); b. all mycorrhizations previously seen in sand-containing supports were contaminations; c. our stored *T. pfeilii* dry fruiting bodies had deteriorated, to the point where spores failed to germinate.

We had earlier reported *T. pfeilii* spore germination on different media using fresh fruiting bodies (Kagan-Zur & al. 1992). We now tried to get spores of our stored samples to germinate under the same and additional combinations. No spores germinated.

We asked for fresh specimens, but none were available during the 1994 season, due to rain failure (see report of the Botswana team). Dried fruiting bodies stored in Botswana since the 1993 season were brought over in November 1994 and tried in both spore germination experiments and inoculation experiments. No germination and no hyphae were observed up to 3 months from inoculation. This means that

spores did not survive our standard storage procedure, namely storage in desiccated containers at room temperature for more than one year.

EXPERIMENTS CONDUCTED IN BOTSWANA

This report covers the experiments which were ongoing as well as those which were planned at the time of writing our last report.

1. Raised bed trial (see Table 4)

Spore inoculation

It was stated in the last report that potted plants would be removed from pots to enable mingling of the roots. Instead plants were left in pots for ease of tracing the roots for identification. Roots from all the four plants species, namely: *Grewia flava, Acacia herbaclada, Acacia melifera* and *Eragrostics tef,* were sampled for observation.

There was mingling of the roots despite having not removed the plastic pots. On observation under the microscope (x 400 magnification), roots of *Grewia flava* had a lot of spores adhering to the roots but not germinating, while a lesser number of spores were observed in *Acacia melifera, A. herbaclada* and *Eragrostis tef.*

Mycelium inoculation

Grewia flava - very thin hyphae were observed but did not look alive.

Acacia melifera - no hyphae

Acacia herbaclada - no hyphae

Eragrostis tef - no hyphae

A lot of contaminants were observed in all control plants. The effect of shading is not quite clear in this experiment. It would seem that the shading was not sufficient to facilitate mycorrhizal formation.

2. *Cynodon dactylon* (2 x 2 m plot)

Ten litres of *Terfezia pfeilii* spores were further inoculated in this plot. Roots were collected (see Fig. 1 for random sampling procedure) for observation. A similar phenomenon was observed as in *Grewia flava.* A lot of spores were observed adhering to the roots but none germinating.

3. Laboratory experiments

Germination of *Stipagrostis uniplumis* and *Schimiditiai pappophoroides* was not successful, while *Eragrostic rigidor* seeds were not available due to drought.

As an initial step toward introduction of Cistaceae plants into the Kalahari, seeds of *Helianthemum sessliflorum, H. ledifolium* and *Cistus salvifolium* were brought in from Israel for trials in Botswana on November 1994 . Truffles are known to favour *Cistaceae* members as their plant symbionts (Chevalier et al., 1975), and symbiosis of several of these with *Terfezia* species was achieved *in vitro* and in pot experiments (Awameh, 1981; Fortas & Chevalier, 1992; Roth-Bejerano et al., 1990).

Four hundred seeds of each specie were surfaced sterilised with household bleach (3.5% sodium hypochlorite) and rinsed with distilled water. These were planted in replicates of a hundred (International Seed Testing Association rules: 1976) in polystyrene containers with moist vermiculite and kept in an incubator at a temperature of 30°C. Germination results were recorded (see Table 5). Five seedlings per species were used as controls while the rest were inoculated with *T. pfeilii* spores three weeks after germination.

No spores were observed on both inoculated and control seedlings.

4. Field trip

A trip to the Kalahari was undertaken in May 1994, but no truffles were found in any of the areas previously visited in our earlier trips, due to erratic rainfall. This has been most unfortunate for our programme.

REFERENCES

Alexander, T., Meier, R., Toth, R. and Weber, H.C. (1988) Dynamics of arbuscule development and degeneration in mycorrhizas of *Triticum aestivum* L. and *Avena sativa* L. with reference to *Zea mays* L. New Phytol. 110:363:370.

Awameh, M. S. (1981). The response of *Helianthemum salicifolium* and *H. ledifolium* to infection by the desert truffle *Terfezia boudieri. Mushroom Science* (Pt 2) **11**, 843-853.

Chevalier, G. and H. Frochot. 1990. Ecologie et possibilité de culture en Europe de la truffe de Bourgogne (*Tuber uncinatum* Ch.) p. 323-330. In: M. Bencivenga and B. Granetti, (eds.) Atti del secondo congresso internazionale sul tartufo. Spoleto, Italy 1988. Com. Mont. dei Martini, Italy.

Chevalier, A, D. Mousain, F. Y. Couteaudier, 1975 Associations ectomycorrlviziennes entre de Tuberaceae et Cistaceae. Ann Phytopathol. 7: 355-356.

Dexheimer, J., Gererd, J., Ledue, J. P. & Chevalier, G. (1985). Etude ultrastrucurale comparee des associations symbiotiques mycorhiziennes *Helianthemum salicifolium - Terfezia claveryi* et *Helianthemum salicifolium - Terfezia leptoderma. Canadian Journal of Botany* **63**, 582-591.

Fortas, Z. and Chevalier, G. (1992). Effet des conditions de culture sur la mycorhization de l'*Helianthemum guttatum* par trois especes des genre *Terfezia* et *Tirmania* d'Algerie. Can. J. of Bot. 70:2453-2460.

Giovannetti, G., Roth-Bejerano, N., Zanini, E. & Kagan-Zur, V. (1994). Truffles and their cultivation. Hort. Rev. 16 (In press).

Hoagland, D.R. & Arnon D.I. (1950). The water culture method for plants without soil. Circular 374, Univ. of California, Agriculture Experimental Station, Berkeley.

International seed Testing Association rules. 1976

Kagan-Zur, V., Dvir, I., Roth-Bejerano, N., Taylor, F. W., & Baker, N. (1992). Cultivation of *Terfezia pfeilii* - the Kalahari truffle. Report no. BGUN-ARI 11-92 Beer-Sheva Israel.

Kagan-Zur, V., Roth-Bejerano, N., Thamage, D., Taylor, F. W., & Baker, N. (1993). Cultivation of *Terfezia pfeilii* - the Kalahari truffle. Report no. BGUN-ARI 12-93 Beer-Sheva Israel.

Kagan-Zur, V., Roth-Bejerano, N., Thamage, D.& Taylor, F. W. (1994). Cultivation of *Terfezia pfeilii* - the Kalahari truffle. Report no. BGUN-ARI 13-94 Beer-Sheva Israel.

Koske R.E. and Gemma J.N. (1989). A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92:486-488.

Mamoun, M. and Olivier, J.M. (1992). Effect of Pseudomonads on colonization of hazel roots by the ecto-mycorrhizal species *Tuber melanosporum* and its competitors. Plant & Soil 139:265-273.

Roth-Bejerano, N., Livne, D. & Kagan-Zur, V. (1990). *Helianthemum-Terfezia* relations in different growth media. *The New Phytologist* **114**, 235-238.

Schwarzel, K. (1967). Beitrag zum Hypogaenflora des Kantons Basel Stadt und seiner naheren Umgebung. Schweiz. *Zeitschrift fur Pilzkunde* **45**, 129-141.

Signorini, D. & Valli, O. (1990). Il tartufo - habitat, ricerca, zone di reperimento, addestramento del cane, tecniche di coltivazione del bianco e del nero. Mistral publ. Milano, Italy.

Table 1. Effect of low pH (2.7-3.0) watering on association between *Cynodon dactylon* seedlings and *Terfezia pfeilii* spores. Soil composition: 70% sand, 25% peat, 5% vermiculite

Dates	
3.1.94	Seeding
11.4.94	Addition of pseudomonas
26.4.94	First pots checked
25-30.5.94	Last check

First check	No. of pots with mycorrhized roots	
(2 pots per treatment)	HCl	H2SO4
Control	2	0
Spores	2	2
Spores + Pseudomonas	2	2
Last check		
(8 pots per treatment)		
Control	0	1
Spores	1	0
Spores + Pseudomonas	3	1

A large number of non germinated spores were observed.
Most of the mycorrhizae were endomycorryzae

Table 2. Effect of support medium on type of mycorrhiza formed between *Terfezia pfeilii* spores and *Cynodon dactylon, Acacia melifera* or a mixture of the two

Medium	Ratio and type of mycorrhizal association		
	Cynodon	Acacia	Cyn.+ Acac.
Sand + 1/2 Hoag.	*6\6 endo	5\5 endo	1\2 endo
Agar 1/2 Hoag	1\2 ecto	2\2 ecto	0\2
Agar 1/2 Hoag, 0.2 P	1\1 ecto	1\2 ecto	2\4 ecto

*No. of mycorrhized pots out of total available at the end of the experiment

Table 3. Effect of support medium on mycorrhiza formation between a putative *Terfezia pfeilii* isolate (mycelium) and *Cynodon dactylon* seedlings

A. Soil versus agar (soil composition as in Table 1)

I. Mycorrhization

Medium	Type of mycorrhizal association Checking date		
	2.2.94	9.2.94	16.2.94
Soil +Hoag.	0\8	1\8 ecto	1\10 ?
Soil + 1/10 Hoag	0\7	0\8	1\8 endo
Agar Hoag	0\8	0\8	0\8
Agar Hoag -P	0\8	0\8	0\8

II. Hyphae presence in the medium

Medium	Fungal outgrowth into the medium Checking date		
	2.2.94	9.2.94	16.2.94
Soil +Hoag.	4\8	5\8	6\10
Soil + 1/10 Hoag	4\8	4\8	6\8
Agar Hoag	0\8	0\8	0\8
Agar Hoag -P	0\8	1\8	0\8

B. Sterile perlite support

I. Mycorrhization

Date	Mycorrhized seedlings		
	1/2 Hoag.	1/2 Hoag. + MES	1/5 Fontana
24.7.94	1/4	0/4	0/4
31.7.94	0/4	0/4	0/3
7.8.94	0/4	0/3	-

II. Hyphae presence in the medium

Date	Presence of hyphae		
	1/2 Hoag.	1/2 Hoag. + MES	1/5 Fontana
24.7.94	2/4	1/4	2/4
31.7.94	1/4	1/4	1/3
7.8.94	1/4	0/3	-

C. *Soil subjected to two sterilization rounds (Soil composition as in TableA)*

I. Mycorrhization

Medium	Type of mycorrhizal association Checking date 3.4.94	12.4.94	20.4.94
Soil +Hoag	0\8	0\7	1\6 endo
" control		0\2	1\4 endo
Soil + 1/10 Hoag	0\8	0\8	0\8
" " control		0\2	0\5
Sand + Hoag	0\8	0\8	0\8
" " control		0\2	0\4
Sand + 1/10 Hoag	0\8	0\7	0\6
" " control		0\2	0\6

II. Hyphae presence in the medium

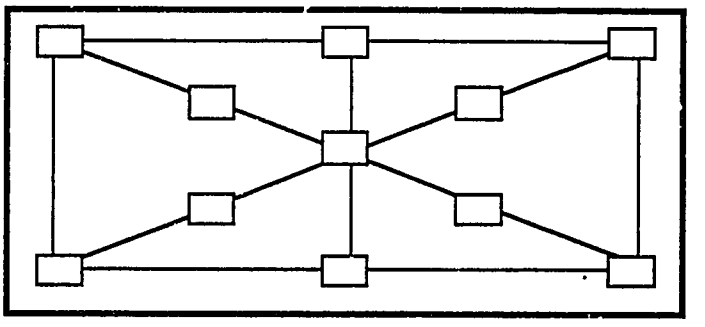
Medium	Fungal outgrowth into the medium Checking date 3.4.94	12.4.94	20.4.94
Soil +Hoag.	1\8	1\8	3\6
" control		0\2	1\4
Soil + 1/10 Hoag	0\8	0\8	0\8
" " control		0\2	0\5
Sand + Hoag	0\8	0\8	3\8
" " control		0\2	1\4
Sand + 1/10 Hoag	1\8	1\8	5\6
" " control		0\2	0\6

Table 4. Mycorrhizae of *Grewia flava, Acacia melifera, Eragrostis tef* and *Acacia herbaclada* in a plot inoculated with spores of *Terfezia pfeilii*

Inoculated species	Shade (25%)			No shade		
	Spores	Mycelium	Control	Spores	Mycelium	Control
G f	III	I	X	III	?	X
A m	I	-	X	-	-	X
E t	-	-	X	I	-	X
A h	I	-	X	I	-	X

Key:
Gf = Grewia flava
Am = Acacia melifera
Et = Eragrostis tef
Ah - Acacia hebaclada
I = Occurence
III = High occurrence
? = Unclear
- = Zero
X = Contaminants

Figure 1. Sampling map of the *Cynodon cactylon* plot



Key: ☐ = sampling areas

Table 5. Results of *H. sessiliflorum, H. ledifolium* and *Cistus salyifolium* germination

REPLICATE	Helianthemum sessiliflorum	Helianthemum ledifolium	Cistus salvifolium
REP I	78	86	96
REP II	67	74	80
REP III	89	51	91
REP IV	93	81	87
TOTAL	327	292	354
Percentage	81.75%	73%	88.5%