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# EFFECT OF SOME SEED TRANAMITTED FUNGI OF BRASSICA NAPUS L. ( RAPE ) ON SEED OIL CONTENT AND FREE FATTY ACID

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## *Abstract*

*Alternaria brassicae* , *Aspergillus flavus, Aspergillus niger* and *Penicillium* sp. were isolated from seeds of rape cv. Semu RT as seed - transmitted fungi.

*Aspergillus flavus* decreased the seed oil content to 37.04 % as compared with 46.68 for healthy seeds indicating about 21 % loss. The remaining fungi, however, showed moderate effect in this respect.

Free fatty acids of seeds varied to different extents according to fungal invasion, where some increased in content , other decreased and some were only found in infected seeds, e.g. margaric, lauric, tridecylic and myrestic acids

## INTRODUCTION

Many species of the family Brassiceae are cultivated for food. Among the most familiar of these is *Brassica napus* L. (rape) . Repeseed oil is nutritious, easy to digest, and is reported to improve the function of the gall bladder. The oil cake , following oil extracton, is rich in protein, nitrogen, phosphorus and potassium. Rapeseed oil is also an important industrial raw material , widely used in producing soap

and paints. It is a good animal feed, and in some countries it is used as an organic fertilizer (Wittwer *et . al* 1987 ) The oil ois also used in the production of synthetic rubber (Ohlson 1972 ).

Repe is commercially planted in some countries including China, India , Canada, France and Poland (Frencel 1983 ). During the recent years, rape was introduced to Egypt as a promising oil crop. It has been grown in some locations in several Governorates, e. g., Behera, Damietta , Fayoum , Giza and Kafr El-Sheikh at different Agricultural Experiment Stations).

Yields of rape can be improved in many ways. among these is to determine and control various fungi causing serious losses in content and quality of seed oil , e. g. *Alternaria* brassicae (Berk.) Sacc., *Aspergillus flavus* (Link ex. Fr.). *Aspergillus niger* (Van Teigh ) and *Penicillium* sp. (Van Schreven 1983 , Rai and Saxena 1980 and Kolte 1983 ). Various reviewers carried out a large number of experiments on diverse pathogens causing serious damage to rape plants and their production of seed oil.

Van Schreven (1953). isolated *Alternaria brassicae* and *A. brassicicola* from rape seeds obtained from plants infected with speckle or dark leaf spot disease where *A. brassicae, A. brassicicola, A. tenuis , Stemphylium spp.* and *Botrytis cinerea* were isolated from necrotic lesions on the stalks and siliques of diseased plants.

Badadoost and Gabrielson (1979) recorded that *Alternaria alternata, A. brassicae, A. brassicicola , Stemphylium spp.* and *Ulocladium* sp. were isolated from dark lesions developed on seeds of some diseased cruciferous plants. Only *A. brassicae* and *A. brassicicola* were pathogenic but the latter was more virulent.

Maude and Humpherson Jones (1980) demonstrated that tests in the UK on samples of basic and commerical *Brassica oleracea* seeds during 1976 - 78 showed that many lots were infected by *Alternaria brassicicola*, however, *A. brassicae* was uncommon in the basic seeds in these years and in commerical seeds harvested in 1987 . Most affected seeds were contaminated by surface - borne spores and mycelium of *A. brassicicola* , but many were internally infected within the seed coat and in some cases in the embryo.

Rai and Saxena (1980) recorded that *Alternaria brassicae, Aspergillus flavus, A. fumigatus* and *Cladosporium herbarum* , isolated from stored *Brassica juncea*

seeds reduced oil content and free fatty acids associated with the increase in the incubation period for all tested fungl.

Humphersom - Jones (1983). showed that *Alternaria brassicicola and A. brassicae* occurred on *Brassica oleracea* and *B. napus during* 1977 - 1980 in which *A. brassicae* developed pod infection.

Humphersom - Jones and Maude (1983) found that seeds obtained from diseases rape were considered as source of inoculum of *Alaternaria brassicae* and *Leptosphaeria maculans* .

Kolte (1983) reported that *Alternaria* blight caused by *A. brassicae* adversely affected oil yield of infected plants.

Tripathi and Kaushik (1984) recorded the association of *Alternaria brassicae* with seeds from three different varieties of rape (*Brassica campestris* ) and *B. juncea* .

The present work was planned to determine the seed-transmitted fungi associated with rape and to elucidate the effect of fungal infection on seed oil content and composition of fatty acids.

## MATERIALS AND METHODS

Seeds of rape, cv . Semu RT 82, were 82 were collected from Giza Governorate at harvest time to isolate and detect seed - borne fungi . Seeds were surface sterilized with 3 % sodium hypochlorite for 5 min ., serially washed in distilled water and carefully dried between two sterile filter papers. Twenty seeds were placed on five layers of moist blotters in Petri dish, 12 cm in diameter and incubated at $22^{O}C$ for 10 days . Samples were examined by stereomicroscepe binocular (400 X). Single spores or hyphal tips were picked and transferred onto PDA slants . Purified cultures obtained from infected seeds were kept on PDA slants. The isolated fungi were identified through the collection of the Mycology Dept., Plant Path. Inst ., Agric . Res . Centre, Giza . Identification was based on morphological and cultural characteristics and according to Gilman (1957) and Barnett and Hunter 1972.

Four Fungi were isolated and identified. They are *Alternaria brassicae* , *Aspergillus flavus, Aspergillus niger* and *Penicillium* sp. Seeds of rape were infested

separately with each of these fungi by soaking in the fungus spore suspension for 24h . Seeds of the control were moistened with sterilized water . Both inoculated and uninoculated seeds were incubated at 25$^{0}$ C for 15 day . The following biochemical constituents were investigated.

**(a). Determination of oil content :**

Dried samples of healthy and infected rape seeds, 5 g each , were extracted for 16 h. in Soxhlet apparatus using petroleum ether (Anon 1980). The extraction was filtered . The filtrate was evaporated at 100$^{0}$ C . The resulting residue was dried to a constant weight . The oil content in proporation to weight of dried seeds was calculated.

**(b). Identification and content of free fatty acids :**

The lipids were extracted from healthy and infected seed samples according to the method given by Blight and Dyer (1959). Physical and chemical analysis of lipids extracted from heathy and infected seeds were carried out . The unsaponifiable matter was determined (Anon. 1975 ). Free fatty acids were extracted according to the method outlined by Borgstrom (1952) before being converted into methyl esters where the solvent was distilled off, then the residue was dissolved in anhydrous diethyl ether and methylated by dropwise addition of diazomethane solution until the yellow colour persists. The mixture was then kept at the room temperature for 15 min. and the solvent was evaporated on a water bath. Finally , the methyl ester of fatty acids were dissolved in chlorofrom and aliquets of this solution were subjected to GLC analysis in order to identify the methylated fatty acids using the technique of Farag *et. al* (1985).

## RESULTS AND DISCUSSION

The oil content of rape seeds (Table 1). was affected to a large extent by fungi transmitted by seeds. Healthy seeds contained 46.68 % oil . *Aspergillus flavus* showed the most harmful effect on the oil content among the studied fungi being 37.04 % . Both *Alternaria brassicae* and *Penicillum* sp. had a moderate effect in this respect where oil contents were 43.70 and 43.60%, respectively . *Aspergillus niger* , however, did not affect the oil content of rape seed being 46.28 %.

Rai and Saxena (1980) working on *Brassica juncea* seeds showed a similar decrease in oil content as a result of *Aspergillus flavus* infection . In the mean time, the same authors as well as Kolte (1983) reported that *Alternatia brassicae* decreased the oil content of seed, being in harmony with the aforementioned findings.

Table 1. Oil content of rape seeds as affected by four fungi transmitted by seeds.

| Fungi | Oil % | Reduction % |
|---|---|---|
| *Alternaria brassicae* | 43.70 | 6.38 |
| *Aspergillus flavus* | 37.04 | 20.65 |
| *Aspergilllus niger* | 46.28 | 0.086 |
| *Pencillium* sp. | 43.60 | 6.59 |
| Healthy control | 46.68 | -- |

Table (2) and Figures (1 to 6) depict the relative retention time (RRT) of free fatty acids relative to oleic acid as well as their content in seeds of rape as affected by tested fungi . Six fatty acids were detected in healthy seeds at various content; oleic acid (77.19 % ) , linoleic acid (13.97 % ) , palmitic acid (5. 35 % ), linolenic acid (2.99 % ) , stearic acid ( 0.33 % ) and palmitoleic acid ( 0.17 % ) . Such contents of fatty acids varied as a result of fungal infecton, where some fatty acids increased in content and others decreased . However, oleic acid was the major component in all treatments . Evenmore, four fatty acids not found in healty seeds were detected in the infected ones.

Infection with *Alternaria brassicae* or *Penicillium* sp. was accompanied by the production of margaric acid at the rate of 1.02 and 0.55 % , respectively. *Aspergillus flavus* and A. niger infection led to the production of lauric and tridecylic acids. Their content was 0. 15 and 0 .19 %., respectively for *A. flavus* and 0.59 and 0.44 % , respectively in case of *A. niger* . Moreover, the latter fungus led to the formation of myristic acid at the rate of 017 %.

Rai and Saxena (1980) found that *Alternaria brassicae* and *Aspergillus flavus* decreased the fatty acids of *Brassica juncea* seeds.

In conclusion the oil content in rape seeds was affected to various extents , due to seed-transmitted fungi. The largest decrease in oil content was caused by*Aspergillus flavus* . Likewise, free fatty acids of rape seed were affected .

Table 1. Identification and content of free fatty acids in seeds of rape as affected by four fungi.

| Fatty acid | Component | R.R. T* Oliec acid | Fatty acids % | | | | |
|---|---|---|---|---|---|---|---|
| | | | *Alternatia brassiccae* | *Aspergillus flavus* | *Aspergillus niger* | *Peicillium* Sp. | Unino-culated seeds (control) |
| C 12 :0 | Lauric acid | 0.33 | 0.00 | 0.15 | 0.59 | 0.00 | 0.00 |
| C13 : 0 | Tridecyic acid | 0.37 | 0.00 | 0.19 | 0.44 | 0.00 | 0.00 |
| C14 : 0 | Myristic acid | 0.43 | 0.00 | 0.00 | 0.17 | 0.00 | 0.00 |
| C16 : 0 | Palmitic acid | 0.61 | 5.72 | 5.65 | 5.83 | 5.26 | 5.35 |
| C16 : 1 | Palmito leic | 0.67 | 0.14 | 0.10 | 0.18 | 0.22 | 0.17 |
| C 1 : 0 | Margaric acid | 0.74 | 1.02 | 0.00 | 0.00 | 0.55 | 0.00 |
| C18 : 0 | Stearic acid | 0.39 | 0.23 | 0.07 | 0.35 | 0.17 | 0.33 |
| C18 : 1 | Oleic acid | 1.00 | 78.76 | 75.41 | 73.62 | 78.00 | 77.19 |
| C18 : 2 | Linoleic acid | 1.16 | 12.10 | 16.02 | 16.96 | 14.40 | 13.96 |
| C18 : 3 | Linoleic acid | 1.49 | 2.03 | 2.41 | 1.86 | 1.39 | 2.99 |

* R. R. T. : Indicates relative retention time and the retention time of 18.1 was given a value of 1.00.

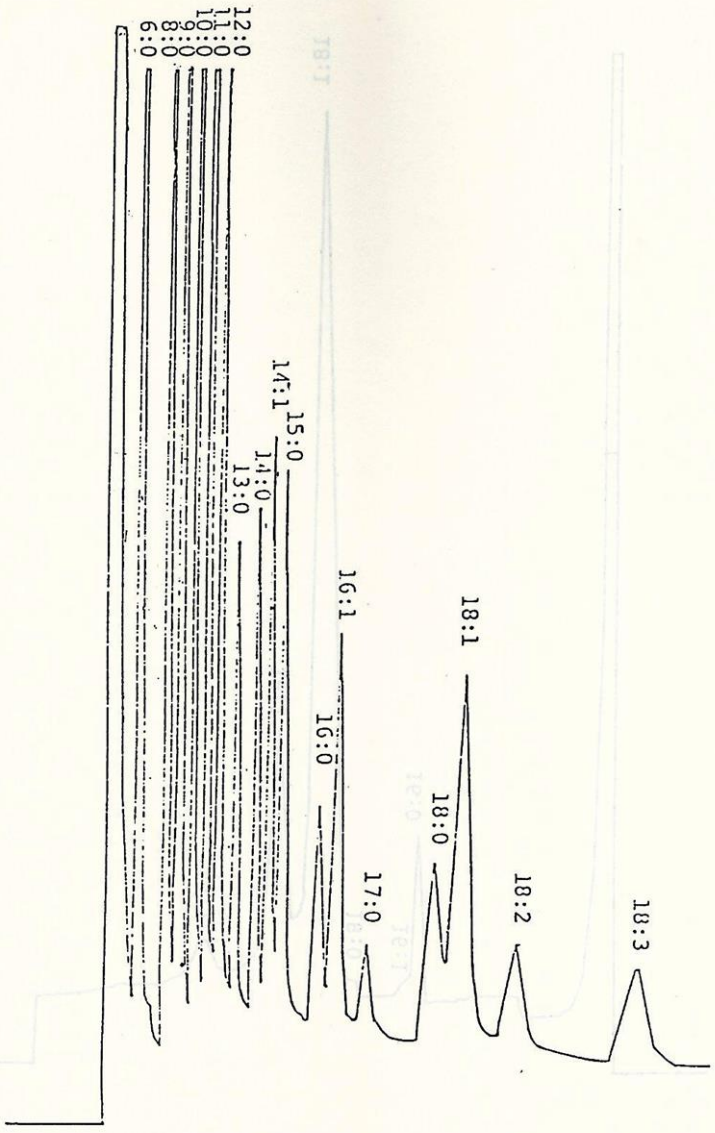


Fig 1. Standard curve of free fatty acids in rapessed

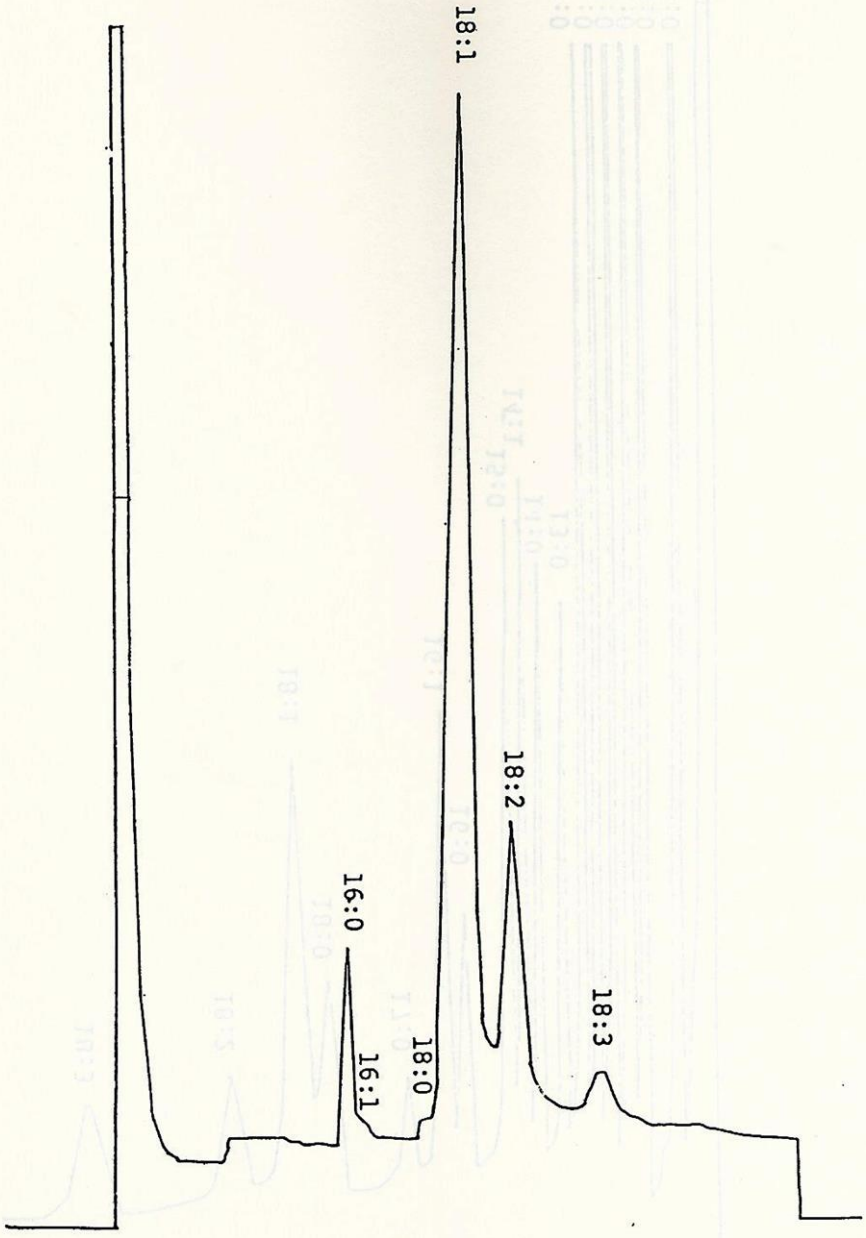


Fig 2. Free fatty acids in healthy rapeseed.

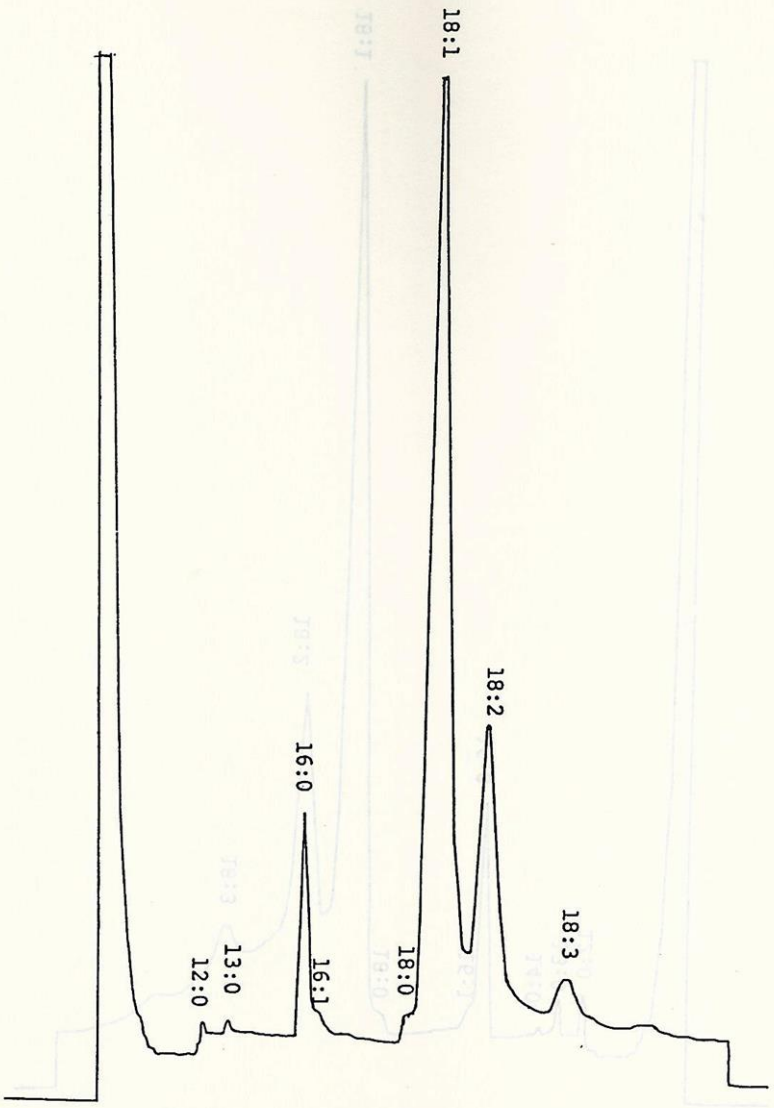


Fig 3. Free fatty acids in rapeseed infected with *Asperquillus flavus* .


Fig 4. Free fatty acids in rapeseed infected with *Asperquillus flavus* .

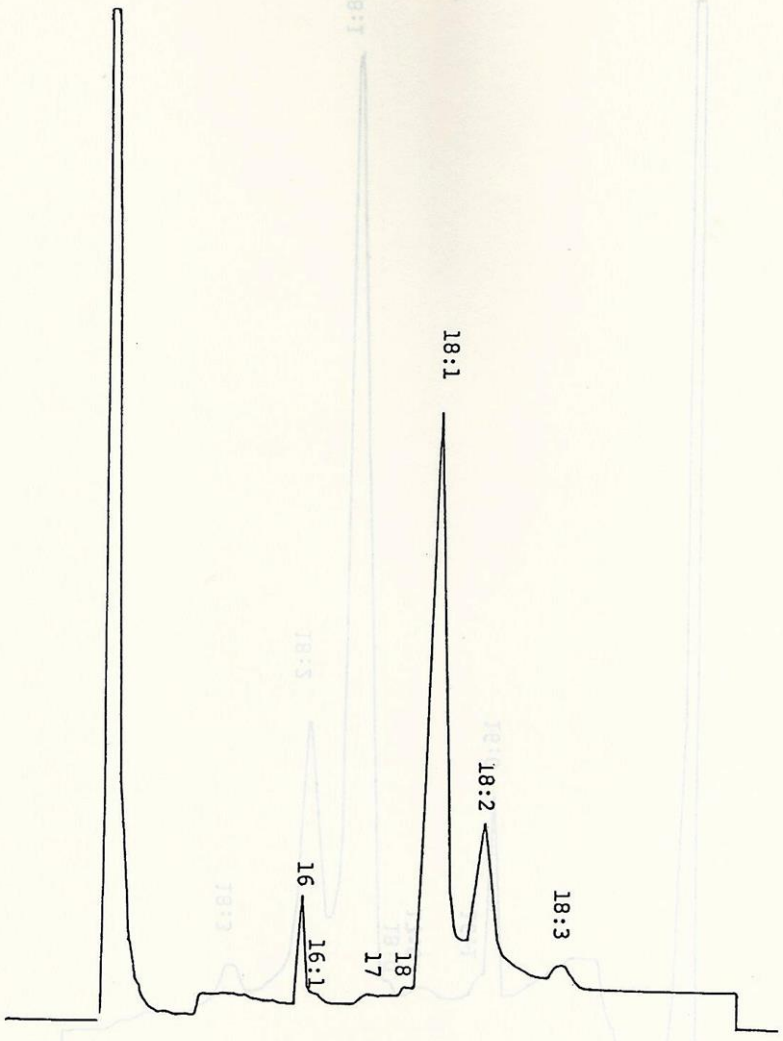


Fig 5. Free fatty acids in rapeseed infected with *Asperquillus flavus* .

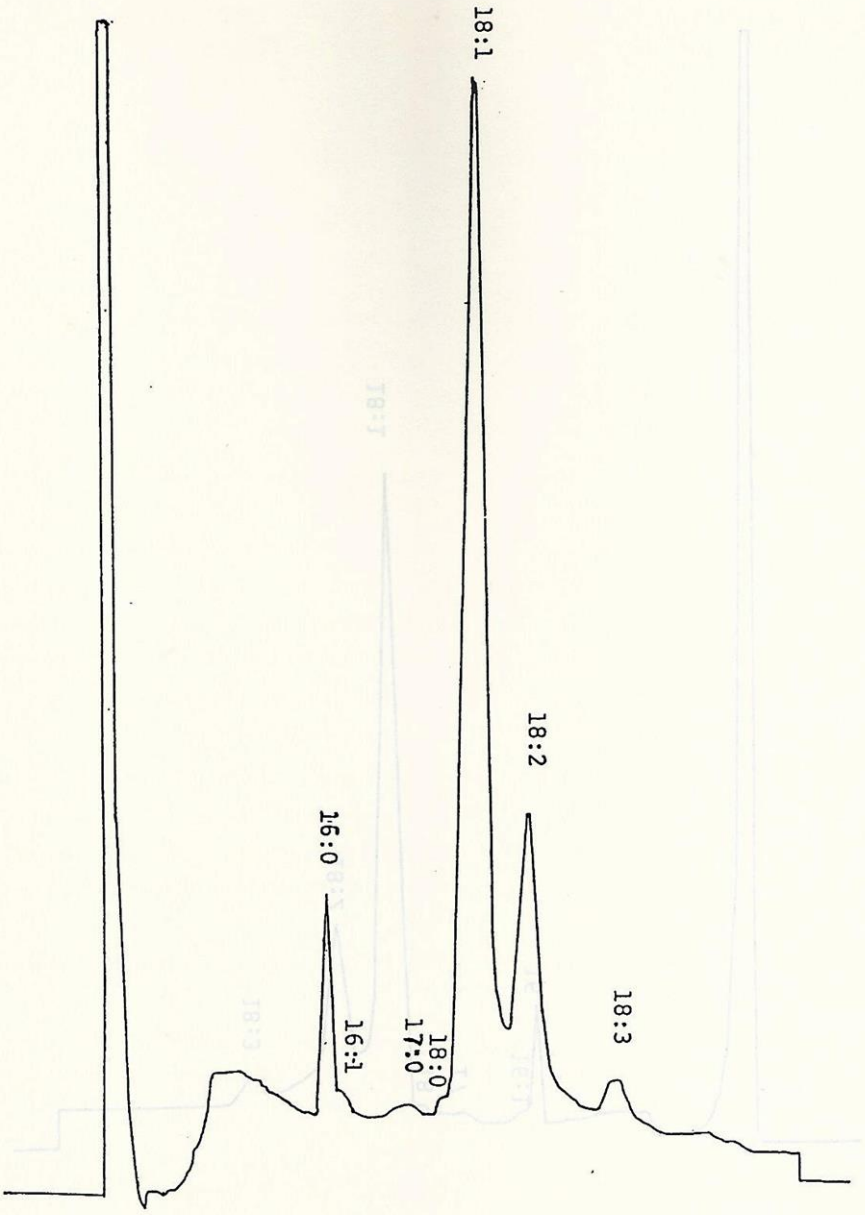


Fig 6. Free fatty acids in rapeseed infected with *Alternaria brassicae*

by seed - transmitted fungi, where some acids increased and others decreased in content and some fatty acids were only detected in infected seeds.

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# تأثير بعض الفطريات المنقولة بالبذور على محتوى الزيت والأحماض الدهنية الحرة بنبات الريب (الشلجم)

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عزلت الفطريات : -

*Penicillum* sp. *Alternaria brassicae* , *Aspergillus flavus* , *Aspergillus niger*,

من بذور نبات الريب الصنف الزراعى Semu RT 82 كفطريات منقولة بالبذرة لدراسة تأثيرها على محتوى الزيت والأحماض الدهنية الحرة .

تسبب الفطر *Aspergillus flavus* فى انخفاض محتوى زيت البذور الى ٣٧ر٠٤ ٪ بالمقارنة بـ ٤٦ر٦٨ ٪ للبذور السليمة ، ولقد كان تأثير الفطريات الأخرى فى هذا الصدد معتدلاً .

استجابت الأحماض الدهنية الحرة للفطريات بدرجات متفاوتة حيث ارتفع محتوى البعض وانخفض محتوى البعض الآخر وتكونت بعض الأحماض فى البذور المصابه فقط هى حامض مرجريك وحامض لوريك وحامض ترای ديسيلك وحامض مرستك .