

## EFFECT OF CERTAIN MEDICINAL PLANT NATURAL PRODUCTS ON *Meloidogyne Incognita* MANAGEMENT On tomato UNDER GREENHOUSE CONDITIONS

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### ABSTARCT

Under greenhouse conditions, the tested materials i.e. plant water seeds extract solutions have significantly reduced and affected number of galls and eggmasses of *Meloidogyne incognita* on roots and its number of J<sub>2</sub> in soil of tomato, *Lycopersicon esculentum* Mill cv. Castle Rock compared to untreated control . Isothiocyanates components from seeds of *Sinapis alba*, *Ammi visnaga*, and *Lepidium sativum* using its biofumigation separately on the degree of nematode reduction parameters varied according to the concentration and type of the tested materials. Significant increase in weight of fruits / plant was also achieved by most tested materials. These results suggested that species of Apiaceae plants may be used in crop rotations as antagonistic plants or green manures to reduce *Meloidogyne* galls and eggs on rot systems. Dry preparations of these tested plants or extracts could be used as bionematicides.

**Keywords :** *Meloidogyne*, medicinal plants ,seeds aqueous extracts, in vivo

### INTRODUCTION

Plant-parasitic nematodes are microscopic soil worms that reduce the yield and quality of many crops. However, many of the conventional nematicides that were used to control plant-parasitic nematodes have been shown to contribute to ground water contamination and to be hazardous to the health of humans and animals ,and have therefore been banned or restricted in use. Brassicaceae plants produce glucosinolates which are  $\beta$ -D-thioglucosides, distinguished from one another by differences in their organic side chains (R groups). Glucosinolates, classified as aliphatic, aromatic or indole forms, occur in all parts of the plant and degrade via enzymatic hydrolysis. As a result of tissue damage, the relatively non-reactive glucosinolates react with myrosinase, which is stored separately in the cell, to yield nitriles, epithionitriles, thiocyanates and isothiocyanates (ITCs) (Borek *et al.*, 1998, Fahey *et. al.*, 2001 and Lazeri *et. al.*, 2004).

The objective of the present investigation was to study the role of Brassicaceae seeds natural product on *Meloidogyne* spp. management under greenhouse conditions.

### MATERIALS AND METHODS

In order to study the influence of the three medicinal plants (i.e. *Sinapis alba*, *Ammi visnaga* and *Lepidium sativum*) either as plant extracts or ground oils seeds on controlling root-knot nematodes *Meloidogyne incognita*

infecting tomato plants under greenhouse conditions. Sixty four plastic pots ( 20 cm d.) containing 4.5 kg sterilized sand soil with one tomato seedling 30 day-old each were used in this study. One week after tomato seedling transplanting, one thousand (1000) *Meloidogyne incognita* second stage juveniles ( $J_2$ ) was introduced to twelve plastic pots. One week later, the three medicinal plants were added as plant extracts at concentrations of plants extraction 120ml for *Sinapis alba* in 1080ml water ,100ml *Ammi visnaga* in1000water and 35ml *Lepidium sativum* in1165water, respectively. The same medicinal plants were used as ground oil seeds at the rate of 2, 4, 6 and 8 g for each treatment. Another four plastic pots with of nematode and without any tested treatments.

Treatments were replicated five times as follows:

1. N + 120ml for *Sinapis alba* in 1080ml water,
2. N + 100ml *Ammi visnaga* in1000water,
3. N+ 35ml *Lepidium sativum* in1165water,
4. N+ *Sinapis alba* (2 g),
5. N+ *Sinapis alba* (4 g),
6. N+ *Sinapis alba* (6 g),
7. N+ *Sinapis alba* (8 g),
8. N+ *Ammi visnaga* (2 g),
9. N+ *Ammi visnaga* (4 g),
10. N+ *Ammi visnaga* (6 g),
11. N+ *Ammi visnaga* (8 g),
12. N+ *Lepidium sativum* (2 g),
13. N+ *Lepidium sativum* (4 g),
14. N+ *Lepidium sativum* (6 g),
15. N+ *Lepidium sativum* (8 g), and
16. N alone.

Plastic pots with tomato seedlings were arranged in a randomized complete block design on a greenhouse bench at  $30\pm 2^\circ\text{C}$ . Plants received water and protected by conventional pesticides against mites and insects as needed. Plants were harvested after 60 days from nematode inoculation. Data dealing with plant length, fresh weights of shoot and root, shoot dry weight were determined and recorded. Infected tomato roots of each replicate were washed with tap water separately fixed in 4% formalin for 24 hr and stained in lactic acid fuchsin (Byrd, *et al.*, 1983) and examined with stereoscopic microscope for the number of galls and eggmasses of *M. incognita* and recorded. Then data on eggs/eggmasses, root galls, eggmasses number per one gram of infected root/replicate of each treatment was calculated and recorded. *M. incognita* ( $J_2$ ) was extracted from soil/ plastic bag in 100g/ replicate through sieving and modified Baermann technique (Goodey, 1957) counted by Hawksely counting under x10 magnification microscope, recorded and calculated for each bag (4.5 kg) soil.

**Table(1):Isothiocyanates,origin,structure, Molecular weight,and concentration of isothiocyanates to *Sinapis alba* ( nematicidal activity).**

Major components of Isothiocyanate	Concentration PPM	Structure of side chain R	Molecular weight
Lucanine 2	14.3	C27H30O16	440
12-octadeca dienoic acid,(Z) -2,3-bis(trimethyl silyl) oxy] propyl ester	12.7	C27H45O4Si2	498
15-Hexa deca methyl-octasiloxane	12.3	C16H50O7Si8	578
13-teradeca methyl-Hepta siloxane	10.4	C14H44O6Si7	504
11-Dodecamethyl-Hexa-siloxane	9.2	C12H38O5Si6	430
15- octadeca trienoic acid,2,3- bis(tri methyl silyl) propyl ester,(z)	8.5	C27H52O4Si2	496
Ethyl isoallocholate	8.4	C26H44O5	436

**Table(2):Isothiocyanates,origin,structure, Molecular weight,and concentration of isothiocyanate to *Ammi visnaga*( nematicidal activity).**

Major components of Isothiocyanate	Concentration PPM	Structure of side chain R	Molecular weight
Ethyl t-5- carbomethoxy-3- dichloro-t-2- methyl cyclo propane- r- thio carboxylate.	56.9	C11H16C12OS	298
8a' -Dicyano- 4,6- dimethyl-7-trioxa tetracyclo (10) dodecane	53.1	C13H14N2O3	246
7H-furo {3,2-g}[1]benzopyran-7-one,2,3-dihydro-2-(1- hydroxyl-1- methyl ethyl	49.2	C14H14O4	246
5H- furo{2-g}{1} benzopyran-5- one 4,a-dimethoxy-7- methyl-	43.8	C14H12O5	260
2- Pentanone,4-hydroxy-4-methyl	40.4	C6H12O2	116
Visnagin	39.3	C13H10O4	230
6 Dimethoxy-2- ethyl benzaldehyde	37	C11O14O3	194
Hydroxy--i dimethoxy-3' -methyl acetophnone	31.4	C15H16O4	260
2-Benzene dicarboxylic acid, bis(2-ethyl-hexyl) ester	28.9	C24H38O4	390

**Table(3):Isothiocyanates,origin,structure, Molecular weight,and concentration of isothiocyanates to *Lepidium Sativum* ( nematicidal activity).**

Major components of Isothiocyanate	Concentration PPM	Structure of side chain R	Molecular weight
4-Benzobicyclo (4.2.0) oct-3-en-2-one	54.2	C12H12O	172
Anhydro 6-(4Methyl phenyl)aminopyrido(1,2-d) quinazolin-7-ium hydroxide	51.1	C19H15N3	285
2- Pentanone, 4hydroxy-4- methyl	47.2	C6H12O2	116
8H-fura(3,4-d) dibenz(b,f) azepine	37.4	C16H11NO	233
10- hydroxyl-1,4,5,8- tetra- methylanthrone	37.4	C18H18O2	266
Dimethyl-bis (1-methyl-2- pyrrolyl) germane	34.6	C12H18GeN2	264
2' Dihydro -1,2',3- triphenyl spiro(2) pyr azoline-4,3'(4', H)- Quinoline) -5one	32.3	C29H23N3O	429
6-Dimethyl-3-(methoxy methyl)-p- benzoquinone	28.6	C10H12O3	180

## RESULTS AND DISCUSSION

In the large -pot (20cm) experiments, three Isothiocyanate components of medicinal plants (ie. *Sinapis alba*, *Ammi visnaga* and *Lepidium sativum*) at a concentration of 2g ,4g ,6g and8g were most effective by reducing root galling . In the large-pot experiments, mixing each of these three Isothiocyanate components into the soil reduced galling indices in tomato roots. Fresh shoot weights of tomato plants grown in untreated soil were significantly less than those grown in Isothiocyanate components - treated sand. The weight of tomato plants grown in untreated soil was 104g (Table 4) was less than those soils treated with Isothiocyanate components of *Sinapis alba*, *Ammi visnaga* and *Lepidium sativum* that averaged 350,458 and 440g (Table 5) respectively. All the Isothiocyanate components at different concentrations of sand reduced galling rating in the large-pot experiment. No galls were found on tomato roots grown in pots treated with *Sinapis alba*, *Ammi visnaga* and *Lepidium sativum* (Tables 6&7). No significant differences were observed in the fresh shoot weight of these tomato plants. Roots of tomato plants grown in Isothiocyanate components treated soil had very few or no galls, while control plants had heavily galled roots. Biofumigation by means of Brassicaceae green manure or seed meal incorporation into soil is a promising, environmentally friendly alternative to chemical fumigation by methyl bromide for the control of soil-borne pathogens. This biological approach is based on the release of glucosinolate-derived toxic compounds, mediated by endogenous myrosinase (E.C. 3.2.1.147) from Brassicaceae disrupted tissues or seed meals, in the presence of water (Brown and Morra, 1997). Glucosinolates are glucosidic compounds characteristic of Brassicaceae. They are important constituents of the defensive system together with the enzyme myrosinase. There are more than 120 glucosinolates in nature and frequently one predominates in a specific plant, tissue or seed (Fahey et al., 2001). Glucosinolates can be aliphatic, thiofunctionalized, aromatic or indolic, determining the biological activity of their hydrolysis derivative. Medicinal plants are generally used in the medical, and food industries, and are thought to be safe compounds for humans, animals, and the environment. However, many of medicinal plants and their components have been evaluated for their nematicidal activity, e.g., the medicinal plants of *Ammi visnaga*, *Sinapis alba* ,and *Lepidium sativum* and their components found in Tables (1, 2 and 3) have nematicidal activity. In soil, relatively higher concentrations of Isothiocyanates components were needed to control *Meloidogyne incognita* In the present study, the Isothiocyanates components(water extracts) showing high nematicidal activity in vivo were from *Ammi visnaga*, *Sinapis alba* ,and *Lepidium sativum* followed from the family brassicaceae (mustrad and garden gress ) & Apiaceae (Khella) . Among the chemotypes of *Sinapis alba* and *Ammi visnaga* higher nematicidal activity as (water extracts) were found in the shelf life Isothiocyanates components in soil are present at higher concentrations than *Lepidium sativum* that is less of shelf life in soil to Isothiocyanates components. A nematicidal activity trend existed for J2s and

eggs in vivo tests, and most natural products that immobilized J2 were also effective at inhibiting hatching. All Isothiocyanates selected from the results of the in vivo tests (plant water extracts) reduced root damage caused by the nematode at higher concentrations (100 mg/kg). Therefore, the nematicidal activity of these medicinal plants must be checked further using sandy soil types that have higher clay and organic matter contents and good aeration. The main components of the ally Isothiocyanates that, revealed nematicidal activity were tested for nematicidal activity in pots as seeds. The main components of Isothiocyanates from seeds application were very effective in J2s (infective stage) immobilization, all developmental stages, and hatching inhibition at concentrations. Isothiocyanates showed excellent nematicidal activity in vitro (Salem *et al.*, 2012) and on tomato crop in pot experiments. Because there was a wide range in toxicity among the isothiocyanates, with some 100 times more toxic than others. Relatively slight structural differences can confer profoundly different nematicidal effects, confirming that biological activity is a function not only of the concentration of the product but also of the chemical properties of the R side chain. For both plant-parasitic nematodes tested, there was no clear relationship between structure or molecular weight of the isothiocyanates and their toxicities (Tables 1, 2 and 3). Isothiocyanate toxicity is reported to increase with increasing volatility and decreasing molecular weight (Lewis and Papvizas, 1971&Lazzei *et.al*,1993). Higher molecular weight isothiocyanates were less toxic in headspace experiments due to their lower volatility, but were more toxic than the lower molecular weight isothiocyanates when dissolved in agar (Sarwar *et.al* 1998). In our studies in sand, two of the highest molecular weight isothiocyanates, benzyl and 2-phenylethyl, were the most toxic to both nematodes. Our experimental design, with limited headspace, may have favored the higher molecular weight isothiocyanates. Aliphatic isothiocyanates are expected to be more toxic to a range of organisms than aromatic isothiocyanates (Borek, *et. al* ,1998). In our study, aromatic isothiocyanates were the most toxic to both nematode species. Our results are similar to those of Sarwar *et.al*, 1998 who found that aromatic isothiocyanates were more toxic to soilborne fungal pathogens than aliphatic isothiocyanates when dissolved in agar. The use of commercially available isothiocyanates allows direct determination of LC50 and LC90 values by eliminating glucosinolate to isothiocyanate conversion and the possible presence of other alternative breakdown products (e.g., nitriles and epithionitriles). Halbrendt, and Jing, 1994 reported that, *Tylenchulus semipenetrans* was more sensitive than *M. javanica* to most of the tested isothiocyanates. Such differences in sensitivity of nematodes have been reported for *C. elegans* and *Xiphinema americanum* Cobb, also, isothiocyanates with small LC values and a steep slope are the most toxic. Under field conditions, (*Ocimum basilicum*), marigold leaves (*Tagetes erecta*), pyrethrum leaves (*Chrysanthemum cinerariaefolium*), neem seeds (*Azadirachta indica*) and China berry leaves (*Melia azedarach*) were tested against the root-knot nematode *Meloidogyne incognita* most tested materials have significantly reduced second stage juveniles of *M. incognita* in soil and roots of eggplant (*Solanum melongena*). Derivative of isothiocyanates are

derived from glucosinolates that occur in a variety of brassicaceae and Apiaceae (Tables 1, 2, 3 and 4). This research demonstrates that only some species of brassicaceae and Apiaceae are appropriate for plant-parasitic nematode management systems. Those species that contain benzyl or 2-enylethyl,methyl isothiocyanates are effective against *Meloidogyne incognita* (Tables 1,2 and 3). Medicinal plants seeds that should be, the glucosinolate precursors to benzyl and 2-phenylethyl,methyl isothiocyanates, respectively, there are containing gluconasturtiin. Sinigrin-containing plants (the glucosinolate precursor to allyl isothiocyanate) . *Brassica juncea* contain significant concentrations of sinigrin. (Fahey, *et.al*, 2001). To achieve consistent and reliable nematode suppression, sufficient brassicaceous material must be applied to provide toxic concentrations of appropriate isothiocyanates and slow released isothiocyanates components as seeds in greenhouse application. for the formation of ITCs, and if insufficient water is present hydrolysis will be reduced (Lazzeri *et al.*, 2004). Despite previous studies indicating that increasing the water content of the soil can increase ITC release in soil (Matthiessen *et al.*, 2004). Actual conversion of glucosinolates to isothiocyanates can range from 10 to 60% depending on chemical structure and environmental conditions . The mechanisms of plant extracts action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation .(Konstantopoulou *et al.*, 1994 & Brown and Morra,1996 & Borek *et al.*, 1997). *Brassica* residues for these amendments in the present study further reduced these pathogenic agents. This reduction may be a cumulative effect of bio-toxic volatile compounds released during the decomposition of the residues at prevalent high soil temperatures (38–42 C) and subsequent microbial antagonism. Sulphur-containing volatile substances are toxic on many fungi (Lewis and Papavizas, 1971). Allyl glucosinolate is one of the predominant glucosinolates in *Brassica* sp. and is generally converted to allyl isothiocyanate (AITC) at a pH of 4.0 or greater. AITC, a volatile compound, is as toxic to fungi as methyl isothiocyanate, an active ingredient in commercial soil fumigants (Vaughan *et al.*, 1993& Borek *et al.*, 1994). The concentration of the AITC was found to be directly related to heating of the soil up to 45C (Gamliel and Stapleton, 1993). However, isothiocyanates were not detected at low temperatures (Lewis and Papavizas,1971). High temperatures available during the crop-free summer period give the residues time to release their bio-toxic volatile compounds. The decomposed materials also enrich the nutrient-deficient sandy soil and conserve moisture. (Ritu Mawar and Satish Lodha, 2002) .However ,all biofumigant materials in both assays significantly decreased *M. incognita* populations in soil, as well as root galling indices in tomato plants . grown in biofumigated soils. Treatment which had a higher root galling index in relation to the control, suggests an inadequate decomposition process of the brassicaceae and Apiaceae byproducts. Results of the root gall ratings indicated that the highest level of variations in damage suppression within and among Brassica species and Khella was decrease in plants water extract and seeds of medicinal plants there is no

galls in roots of tomato plants compared to control with out *Brassica* and *Apiaceae* treatments compared to the un amendments control.

**Table(4):Effect of incorporation of selected biofumigation enhanced plant water extract on *Meloidogyne incognita* egg production and root galling on to tomato plant cv. Castle Rock in greenhouse conditions .**

Treatment (Extract)	*Plant growth response						*Parameters of <i>Meloidogyne incognita</i>		
	Biomass (g.)			Plant Length (cm)		Yield of tomato fruits	No of galls per one g. root	No of eggs Per one g. root	**Root gall rating
	Shoot fresh (wt.)	Shoot dry (wt.)	Root (wt.)	Shoot (cm)	Root (cm)				
<i>Sinapis alba</i>	118	16	42	42	21	160	0	0	0
<i>Ammi visanaga</i>	88	12	56	33.8	39	144	6	3000	1
<i>Lepidium sativau</i>	132	16	48	42.8	28.5	180	25	12500	3
N alone	72	18	32	40.5	9.8	104	50	25000	4

N= 1000 J2 of *M. incognita* / plant

\*Each value is a mean of five replicates.

\*\* Root gall index (RGI) or eggmass index (EI): 0= no galling or eggmasses, 1=1-2 galls or eggmasses; 2=3-10 galls or eggmasses; 3= 11-30 galls or eggmasses; 4= 31-100 galls or eggmasses and 5= more than 100 galls or eggmasses. .( Talyor and Sasser,1978).

**Table(5):Effect of incorporation of selected biofumigation enhanced plant Seeds *Meloidogyne incognita* egg production and root galling on tomato as well as tomato yield in greenhouse conditions .**

Treatment (Extract)	*Plant growth response						*Parameters of <i>Meloidogyne incognita</i>		
	Biomass (g.)			Plant Length (cm)		Yield of tomato fruits	No of galls per one g. root	No of eggs Per one g. root	**Root gall rating
	Shoot fresh (wt.)	Shoot dry (wt.)	Root (wt.)	Shoot (cm)	Root (cm)				
<i>Sinapis alba</i>	300	36	50	54.7	26.3	350	0	0	0
<i>Ammi visanaga</i>	398	24	60	45.7	28.5	458	0	0	0
<i>Lepidium sativau</i>	370	54	70	64.5	24	440	0	0	0
N alone	72	18	32	40.5	9.8	104	50	25000	4

N= 1000 J2 of *M. incognita* / plant

\*Each value is a mean of five replicates.

\*\* Root gall index (RGI) or eggmass index (EI): 0= no galling or eggmasses, 1=1-2 galls or eggmasses; 2=3-10 galls or eggmasses; 3= 11-30 galls or eggmasses; 4= 31-100 galls or eggmasses and 5= more than 100 galls or eggmasses. .( Talyor and Sasser,1978).

**Table (6): Effect of medicinal plants extract (BioNem) on infestation of *Meloidogyne incognita* to tomato plant under the greenhouse conditions**

BioNem/pot (g)( Extract)	*Nematode parameters					
	Galls/root	Reduction in galls over IC (%)	Final nematode Population J <sub>2</sub> in soil	Reduction in final population over IC (%)	Number of eggs /10 egg mass	Reduction in number of eggs over IC (%)
<i>Sinapis alba</i>	0	100	0	100	0	100
<i>Ammi visanaga</i>	6	84	504	99.9	1860	66.6
<i>Lepidium sativau</i>	25	50	3000	71.5	3000	46.2
N alone	50	-	10500	-	5580	-

N= 1000 J2 of *M. incognita* / plant

\*Each value is a mean of five replicates.

**Table (7): Effect of medicinal plants seeds (BioNem) on infestation of *Meloidogyne incognita* in tomato plant in the greenhouse conditions**

BioNem/pot (g)( Seeds)	*Nematode parameters					
	Galls /root	Reduction in gall over IC (%)	Final nematode population	Reduction in final population over IC (%)	Number of eggs /10 egg mass	Reduction in number of eggs over IC (%)
<i>Sinapis alba</i>	0	100	0	100	0	100
<i>Ammi visanaga</i>	0	100	0	100	0	100
<i>Lepidium sativau</i>	0	100	0	100	0	100
N alone	50	-	10500	-	5580	-

N= 1000 J2 of *M. incognita* / plant

\*Each value is a mean of five replicates.

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تأثير المنتجات الطبيعية لبعض النباتات الطبية علي مكافحة نيماتودا تعقد الجذور  
"ميليدوجيني انكوجنيتا" علي الطماطم تحت ظروف الصوبة.  
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تم تحت ظروف الحقل دراسة تأثير النباتات الطبية لكل من الخردل وحب رشاد و الخلة الطبي على نيماتودا تعقد الجذور الميلودوجيني انكوجنيتا على نبات الطماطم بطريقتين ومعرفة تأثير كل طريقة كالتالي :  
أ - استخدام مستخلصات النباتية الطبية على نيماتودا تعقد الجذور الميلودوجيني انكوجنيتا على نبات الطماطم بتركيزات (120 مل) مذاب في ماء بمقدار، ( ١٠٨٠ مل)، (٣٥ مل في ١١٦٥ مل ماء) و (١٠٠ مل في لتر ماء) على التوالي، لكل من الخردل وحب رشاد والخلة الطبي على التوالي، ومعرفة تأثير مركبات الأيزوثيوسينيد على الأطوار المختلفة لنيماتودا تعقد الجذور والممثل في عدد العقد الجذرية لجذور نباتات الطماطم وتأثير ذلك على نمو النباتات ، وقد كان أعلى نسبة خفض لعدد العقد الجذرية والطور البرقي الثاني وذلك تبعاً لفهرس العقد الجذرية يكون عند استخدام مستخلص الخردل (صفر) و ثم الخلة الطبي (واحد) ومن ثم يليه حب رشاد (ثلاثة) ، و أيضاً زيادة في محصول الطماطم وذلك بعد شهرين من المعاملة ومقارنة ذلك بنباتات الطماطم الغير مقاومة حيويًا.  
ب- استخدام بذور النباتات الطبية باستخدام طريقة الخلط على نيماتودا تعقد الجذور الميلودوجيني انكوجنيتا على نبات الطماطم بتركيزات (٢ جم) ، (٤ جم) ، (٦ جم) و(٨ جم) لكل من الخردل وحب رشاد والخلة الطبي على التوالي، ومعرفة تأثير تحلل مركبات الأيزوثيوسينيد في التربة على الأطوار المختلفة لنيماتودا تعقد الجذور والممثل في عدد العقد الجذرية لجذور نباتات الطماطم وتأثير ذلك على نمو النباتات ، وقد أوضحت نتائج الدراسة على قدرة جميع النباتات بتركيزاتها المختلفة على خفض الكثافة العددية لنيماتودا تعقد الجذور في التربة وزيادة في نمو النباتات وزيادة وزنها و عدد الثمار وذلك بعد شهرين من المعاملة ومقارنة ذلك بنباتات الطماطم الغير مقاومة حيويًا .  
١- أثبتت الدراسة على ان الخلة الطبي كفاتة كامبيد حيوي أمن على البيئة في مكافحة النيماتودا تعقد الجذور في التربة .  
٢- اثبتت هذه الدراسة ان الطريقة المستخدمة في مكافحة النيماتودا(الخلط و التطهير للتربة ) تعتبر اتجاة جديد يزيد من كفاءة فعالية المبيدات على النيماتودا سواً عالية الفاعلية او منخفضة الكفاءة.

قام بتحكيم البحث

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