Alleviation of phytotoxicity of fensulfothion (Dasanit) on pea

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Received on November 14, 1977 ; Revised on March 8, 1978

Abstract

The germination and early growth of pea, particularly, secondary root development, were highly inhibited by fensulfothion (Dasanit), an organophosphorous pesticide. Various light treatments like red, far-red and white light (18 hr light and 6 hr dark cycle) as well as different phytohormones and acetylcholine treatment had no influence on the seed germination either independently or in combination with each other, nor could these treatments reverse the fensulfothion induced toxicity. On the other hand, the development of shoot, tap root and secondary roots were variously affected by these factors and there was significant interaction among the different treatments. The most severe effect of the pesticide was on the development of secondary roots and often simultaneous application of phytohormones enhanced the toxic effect of fensulfothion. The statistical analysis of the data showed that the interaction among the light, the hormones and pesticide were found to be highly significant particularly, in acetylcholine treatment, where maximum interactions among light, acetylcholine and, fensulfothion were noticed with respect to growth and development of pea seedlings.

Key words : Pea, fensulfothion, red light, far-red light, phytohormones, acetylcholine, germination and early growth.

1. Introduction

A number of pesticides, including organophosphorous compounds, have been often found to be phytotoxic. But, the biochemical basis of such toxicities except in the case of herbicides is not known. It has been reported, in many cases, that the growth inhibition caused by growth retardants, like AMO-1618, phosfon-D, CCC, B995 and EPTC can be counteracted by the application of gibberellic acid[1–5]. The influence of gibberellic acid (GA) and growth retardants in altering the growth of plants are suggested to be mutually antagonistic. Apart from GA, mild reversal of the action of CCC was obtained by the application of choline, betaine and adenine [6]. The inhibition of the growth of wheat seedlings by CCC could be reduced strongly by the root application of acetylcholine[7]. Thus, many chemicals, in addition to GA, totally unrelated structurally, were found to be antagonistic to growth retardants. However, there are very few reports available on the alleviation of phytotoxicity of organophosphorous pesticides by phytohormones[8,9].

The phytotoxicity of organophosphorous pesticides attains interesting biochemical significance in view of the fact that, in recent years, acetylcholinesterase has been located

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in a number of plants[10–19] and acetylcholine (ACh) has been implicated in many phytochrome or phytohormone mediated processes[20–23]. It is well known that organophosphorous compounds exert their action by inhibiting acetylcholinesterase in animals. Studies on acetylcholinesterase from pea revealed that one of the major biochemical targets for fensulfothion (organophosphorous pesticide) phytotoxicity, is also acetylcholinesterase[18, 24].

Phytochrome and phytohormones are the important factors which regulate the growth of plants[21, 25–27]. Since this regulation is altered by pesticides and growth retardants, it is obvious to expect strong interactions between these factors in affecting the plant growth. In the present study, the effect of fensulfothion [*o*, *o*-diethyl (*p*-methyl-sulfinyl-phen, l) phosphorothioate], an organophosphorous pesticide widely used for nematode control, on the germination and early growth of pea has been studied. Attempts were also made to find out whether plant hormones or different light treatments like, red, far-red or white light alter the pesticide action. The results of these studies are presented in this paper.

2. Experimental

Materials

Gibberellic acid (GA), indole acetic acid (IAA), kinetin and acetylcholine were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. and fensulfothion (95% technical grade) from Bayer (India) Ltd., Bombay, India.

Source for light treatment : The light source used was 60 W tungsten lamp. The filters, Red-2444, FRF-700 and Green-2092 were used for red (660 nm), far-red (730 nm) and green (520 nm) lights respectively. The filters were obtained from Rohm and Hass Chemical Co., U.S.A. The light from tungsten lamp was filtered through 10 cm of water layer and then through the corresponding filter which was kept at a distance of 10 cm from the petri plates containing surface sterilized seeds. The temperature was maintained at 25° C throughout the studies.

Methods

Effect of fensulfothion on seed germination : The surface sterilized seeds were treated with different concentrations of fensulfothion (0, 25, 50, 75 and 100 μg/ml). One set of seeds was germinated in total darkness and another set of seeds was exposed to 18 hr light and 6 hr dark cycle, referred hereafter, as white light treatment. Percentage germination was recorded at the end of 48 hr.

Influence of light, hormones and fensulfothion on germination and early growth of pea : In order to see whether red (660 nm) and far-red (730 nm) lights have any influence on seed germination and growth, the pea seeds were exposed to red (R) or far-red (FR) illumination for 10 min, immediately after fensulfothion addition to surface sterilized

seeds. The concentration of fensulfothion used was 50 μg/ml. After light treatment, the seeds were germinated in total darkness.

Seeds germinated under complete darkness without any light treatment and the seeds exposed to white light were also used in the study.

To investigate whether any of the phytohormones or ACh have any influence on the fensulfothion treated and untreated seeds, the seeds were treated with different concentrations of GA, IAA, kinetin (0, 0·1, 1, and 10 μg/ml) or ACh (0, 0·1, 1, 10 and 100 μg/ml) immediately after fensulfothion treatment and before light treatment of surface sterilized seeds.

The percentage germination was recorded after 48 hr. On the 10th day of growth, the lengths of shoot and primary roots of the seedlings were measured. The number of secondary roots was counted for each plant.

In the case of dark, R and FR treatments, all observations and measurements were carried out in the dark room under dim green safe light (520 nm).

Statistical analysis : The data were statistically analysed following factorial design for three factors, with unequal number of samples in each treatment, according to the method suggested by Moroney[28] *and* Snedecor and Cochran[29].

3. Results

Effect of fensulfothion on pea seed germination : Germination of seeds was inhibited in the presence of fensulfothion and the inhibitory effect increased with the pesticide concentration (Table I). Germinating the seeds in total darkness or in the light had similar effect on the germination, both in the presence and absence of fensulfothion.

Table I

Effect of fensulfathion on germination of pen seeds *

Concentration**	Dark	White
0	93·3	95
25	86·0	79
50	73·0	79
75	71·0	58
100	31·0	48

* Mean percentage of germination.
**Concentration of fensulfothion in μg/ml.

Influence of light, phytohormones and ACh on fensulfothion treated and untreated seed germination : Germinating the seeds in total darkness or in white light had no effect on germination as mentioned earlier. Similarly, illuminating the seeds with R or FR light for 10 min after soaking had no significant effect, indicating that pea seeds are non-photoblastic. Different concentrations of the hormones too had no significant influence on the pea seed germination.

Fensulfothion inhibited seed germination was not influenced by any of the light treatments. The effect of various hormones at different concentrations on the fensulfothion toxicity revealed that they had no effect.

Effect of light, hormones and fensulfothion on the growth and development of pea seedlings

Shoot growth : Effect of different hormones and different light treatments on the shoot growth of both fensulfothion treated and untreated seedlings are detailed in Figs. 1, 2, 3 and 4.

Effect of light on shoot growth : Exposure of seeds to white light resulted in the retardation of the elongation of shoot in comparison to the effect of total darkness, R or FR light treatments (Figs. 1, 2, 3 and 4).

Effect of light and hormones on shoot growth : Treatment of seeds with different concentrations of GA had no significant effect on the shoot growth of pea seedlings that were exposed to FR and those grown in total darkness. But shoot growth was retarded in R illuminated seedlings and stimulated in those exposed to white light, at all concentrations of GA tried (Fig. 1). In the case of seedlings that were exposed to white, R or FR, IAA had no effect at lower concentrations, but at higher concentrations it reduced the growth of shoot. However, inhibition of elongation of shoot was observed at all concentrations of IAA in dark grown seedlings (Fig. 2). Kinetin had no significant effect on the growth of shoot in FR or white light exposed seedlings. But it retarded the growth in the case of seedlings exposed to R or darkness at high concentration of kinetin (Fig. 3). Acetylcholine elicited no significant effect on the shoot growth at any of the concentrations tried, both in dark grown and light exposed seedlings (Fig. 4).

Effect of light and fensulfothion : Fensulfothion treatment inhibited the elongation of shoot both in light exposed and dark grown seedlings (Figs. 1, 2, 3, and 4).

Effect of light, hormones and fensulfothion on shoot growth : Gibberellic acid treatment along with different light treatments had no significant effect in reversing the fensulfothion toxicity (Fig. 1). Indole acetic acid, along with different light treatments, had no effect in alleviating the fensulfothion toxicity. In fact, the toxicity was accentuated at high concentration of IAA in dark, white and FR exposed seedlings and was severe at all concentrations of IAA in R illuminated seedlings, except at 10 μg/ml concentration (Fig. 2). Kinetin had no significant effect on the reversal of fensulfothion toxicity both

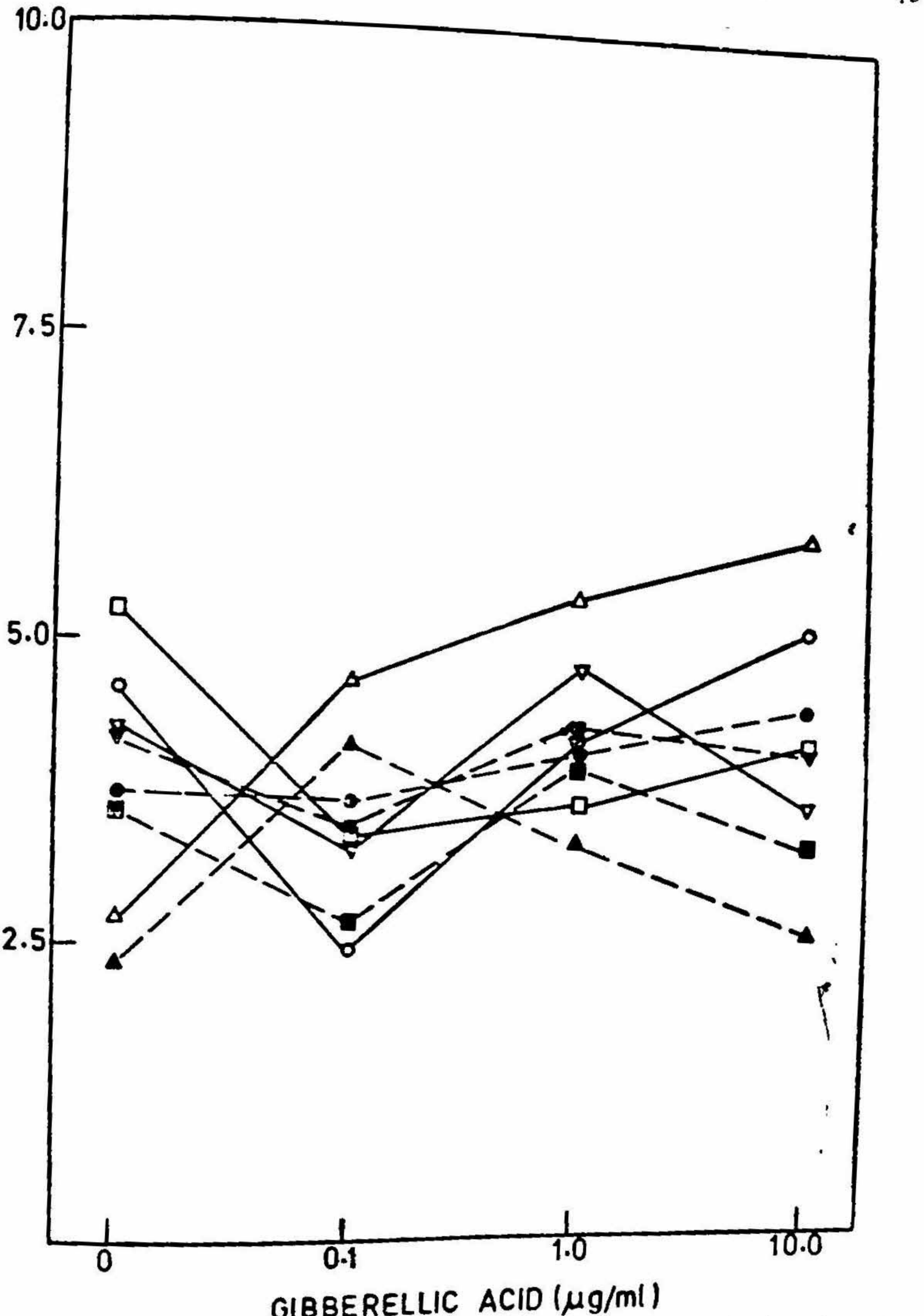

FIG. 1. Effect of light, fensulfothion and gibberellic acid on the development of shoot in pea. (Straight lines with open figures—without fensulfothion; broken lines with closed figures—with fensulfothion; ○—dark; △—white; □—red; ▽—far-red. Least significant difference = 1·54 cm.)

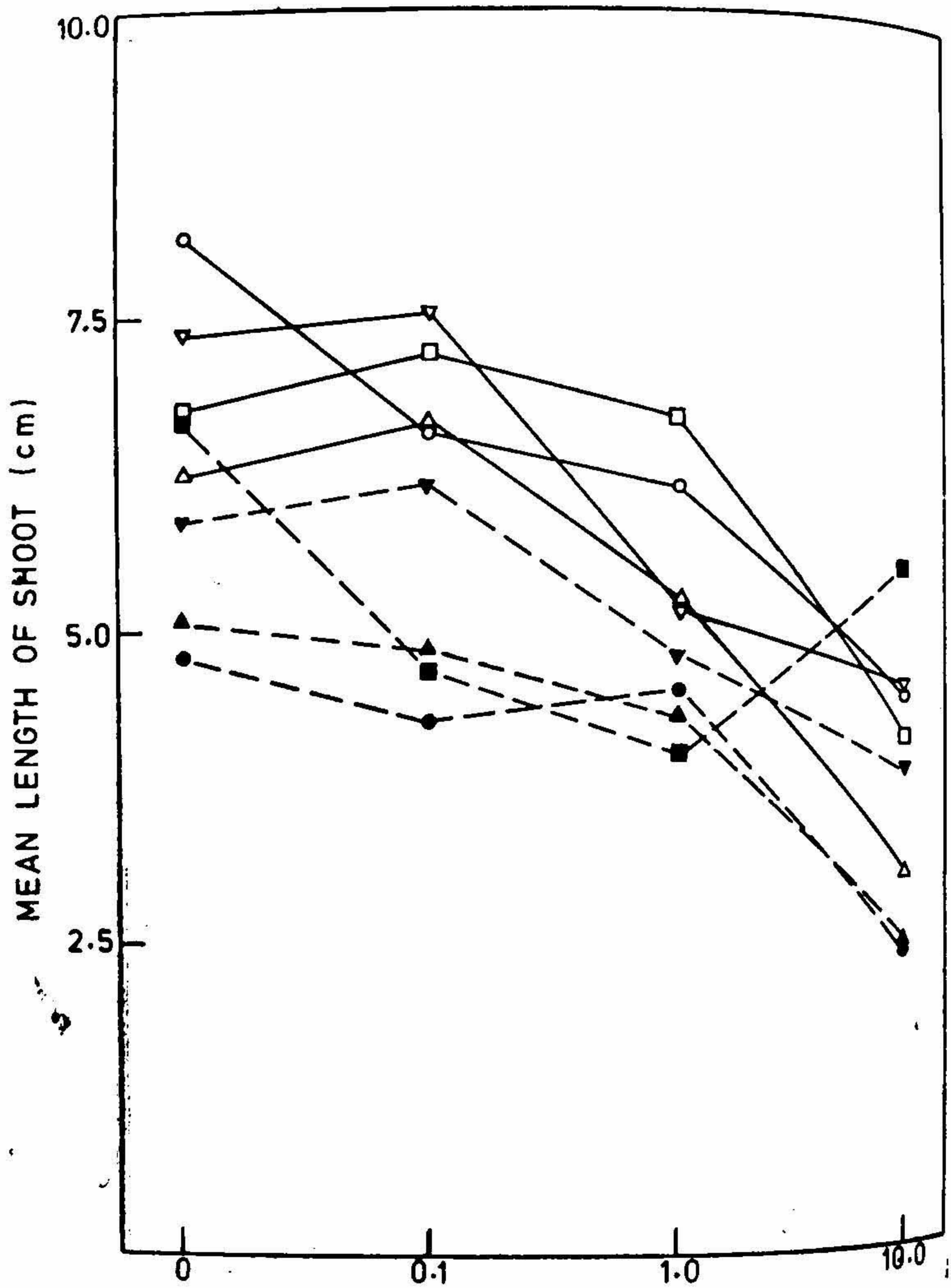

FIG. 2. Effect of light, fensulfothion and indole-3-acetic acid on the development of shoot in pea. (Straight lines with open figures—without fensulfothion; broken lines with closed figures—with fensulfothion; O—dark; Δ—white; □—red; ▽—far-red. Least significant difference = 1·17 cm.)

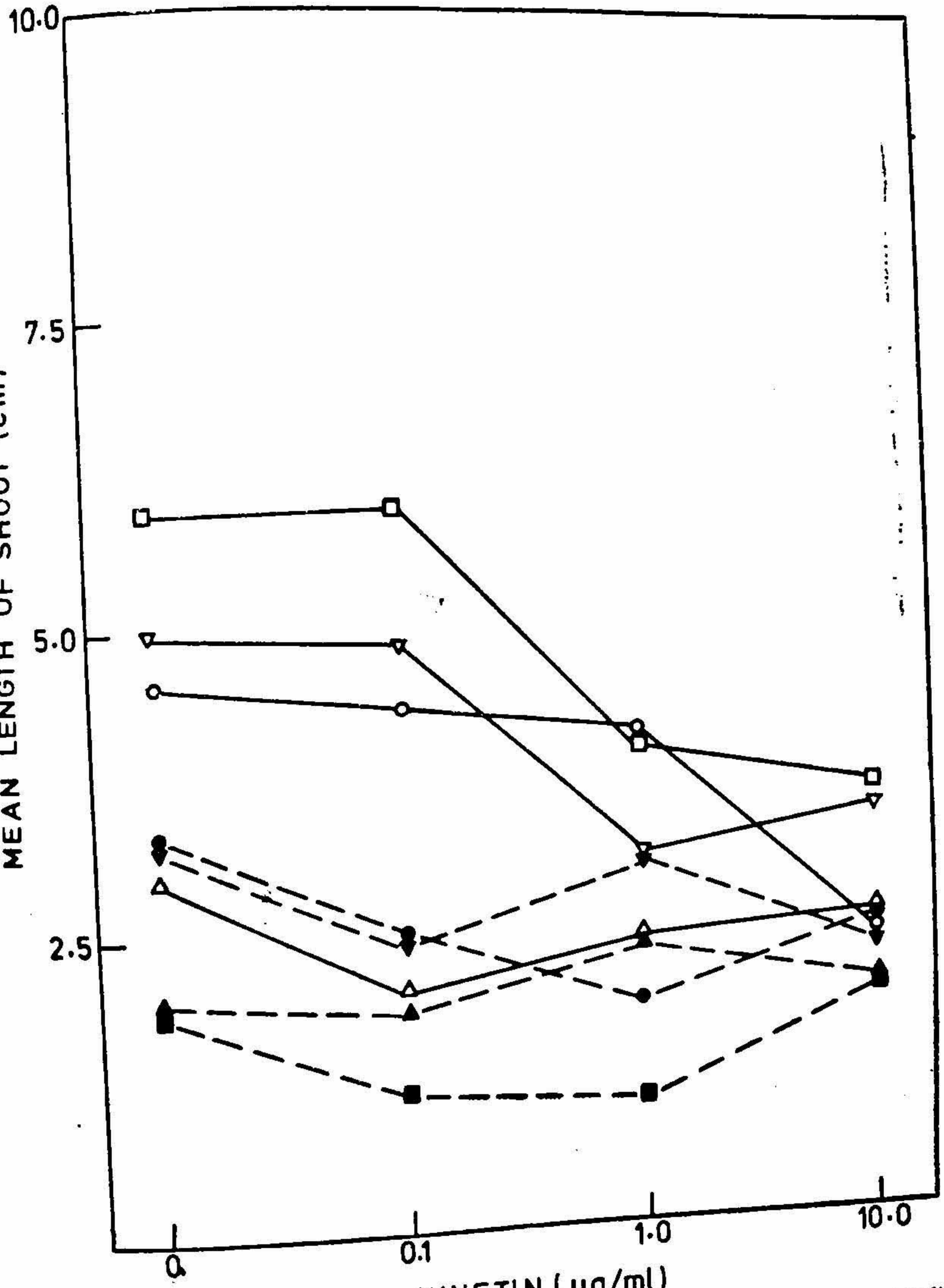

FIG. 3. Effect of light, fensulfothion and kinetin on the development of shoot in pea. (Straight lines with open figures—without fensulfothion; broken lines with closed figures—with fensulfothion; O—dark; △—white; □—red; ▽—far-red. Least significant difference = 1·72 cm.)

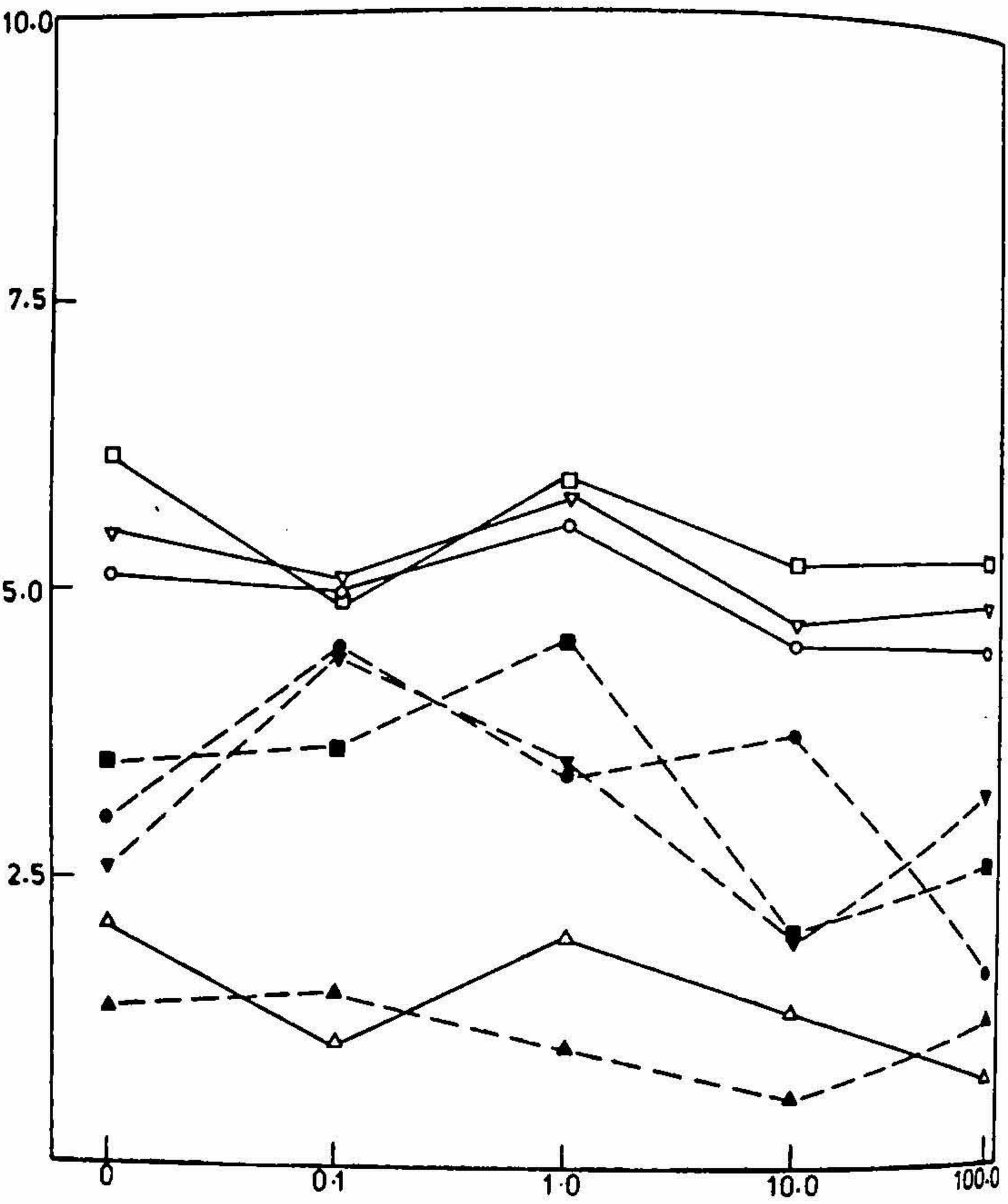

FIG. 4. Effect of light, fensulfothion and acetylcholine on the development of shoot in pea. (Straight lines with open figures —without fensulfothion ; broken lines with closed figures—with fensulfothion ; O—dark ; Δ—white ; □—red ; ▽—far-red. Least significant difference = 1·47 cm.)

in dark grown and light exposed seedlings (Fig. 3). High doses of ACh accentuated the fensulfothion caused growth inhibition in R and dark grown seedlings. But there was a slight improvement in growth at a concentration of 0·1 µg/ml in dark grown and FR irradiated seedlings and at 1·0 µg/ml in R illuminated seedlings (Fig. 4). In the case of seedlings exposed to white light, there was no significant effect of ACh on fensulfothion induced toxicity.

Growth of primary root

Effects of different hormones at various concentrations and different light treatments on the growth of primary root in fensulfothion treated and untreated pea seedlings were investigated. The results obtained are presented in Figs. 5, 6, 7 and 8.

Effect of light on primary root growth : Even the elongation of primary root was retarded when the white light treatment was given, in comparison to the effect of total darkness, R or FR exposure.

Effect of light and hormones on primary root growth : It was observed that high concentrations of GA was deleterious to root elongation both in dark grown and light exposed seedlings in the absence of fensulfothion. It was found to be highly significant in the case of dark grown and FR illuminated seedlings (Fig. 5). Even IAA application caused the retardation of primary root growth especially at high concentration (10 μg/ml) and was more pronounced in the case of FR illuminated seedlings (Fig. 6). Kinetin also reduced the growth of primary root and it was highly significant in the case of R illuminated seedlings, particularly, at high concentration (10 μg/ml) as shown in Fig. 7. Acetylcholine appeared to slightly enhance the root growth at very low concentration (0·1 μg/ml) in dark grown, R and FR illuminated seedlings. But increasing concentrations proved less effective in reversing the fensulfotnion induced toxicity. But in the case of white light exposed seedlings, all concentrations of ACh were inhibitory (Fig. 8).

Effect of light and fensulfothion on primary root growth : Fensulfothion treatment inhibited the elongation of primary roots both in light exposed and dark grown seedlings.

Effect of light, hormones and fensulfothion on primary root growth : Application of GA to the fensulfothion treated seedlings was found to stimulate the primary root growth. However, high concentration of GA was deleterious to root elongation (Fig. 5). Indole acetic acid treatment failed to overcome fensulfothion induced toxicity at all concentrations, under different light conditions (Fig. 6). In the presence of kinetin, the primary root growth in pesticide treated seedlings was affected differently depending on the concentration and the light treatment given (Fig. 7). However, there was no reversal of fensulfothion induced toxicity by kinetin. Acetylcholine treatment slightly enhanced the root growth at very low concentration (0·1 μg/ml), but increasing doses of ACh were less effective in reversing the fensulfothion toxicity.

Formation of secondary roots

Influence of fensulfothion on the formation of secondary roots of pea seedlings exposed to different light treatments as well as to different concentrations of hormones was studied and the results are shown in Figs. 9, 10, 11 and 12.

Effect of light on secondary root formation: In the absence of pesticide or any hormone, there appeared to be no significant difference in the number of secondary roots

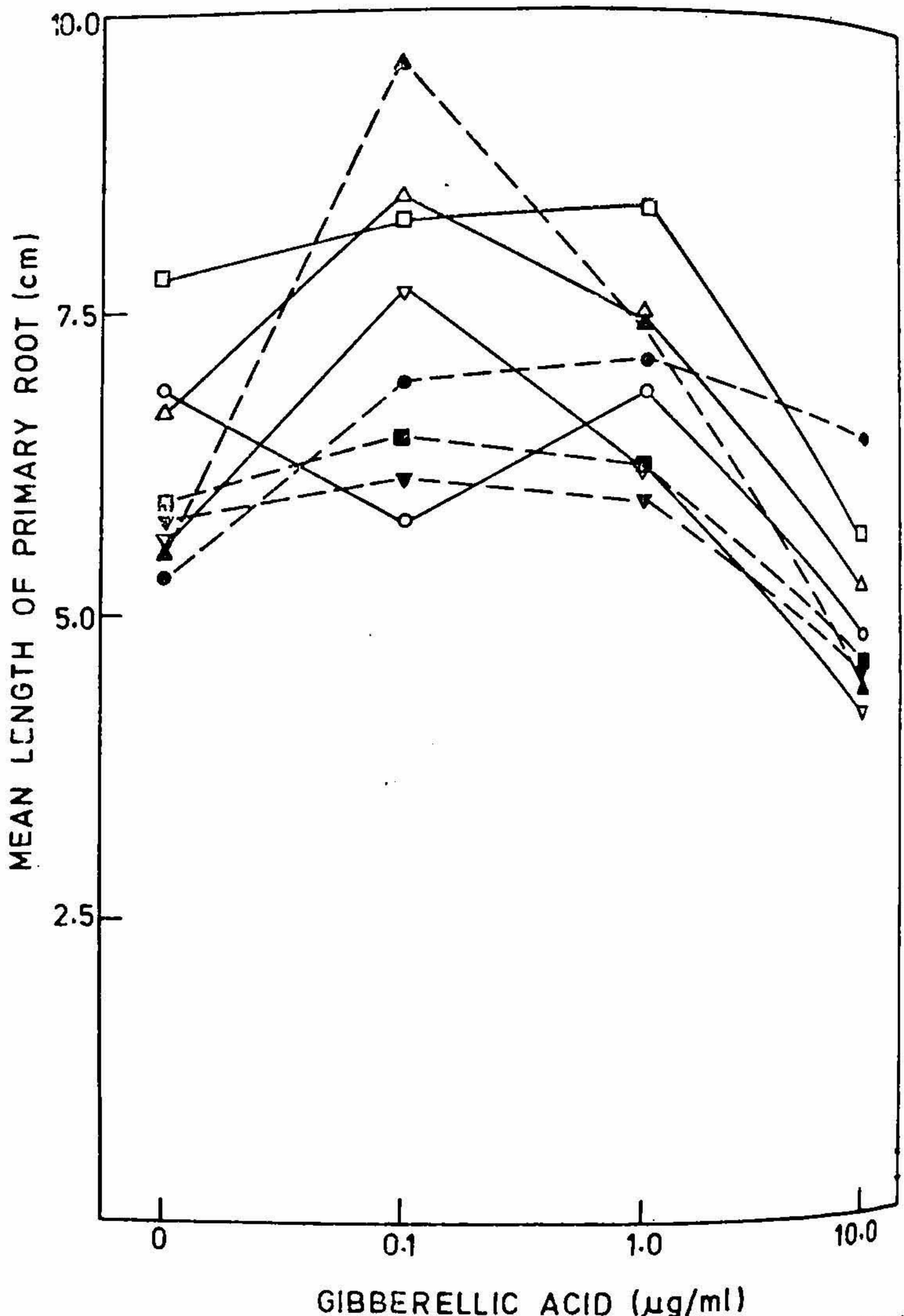

FIG. 5. Effect of light, fensulfothion and gibberellic acid on the development of primary root in pea. (Straight lines with open figures—without fensulfothion; broken lines with closed figures—with fensulfothion; O—dark; Δ—white; □—red; ▽—far-red. Least significant difference = 2·16 cm.)

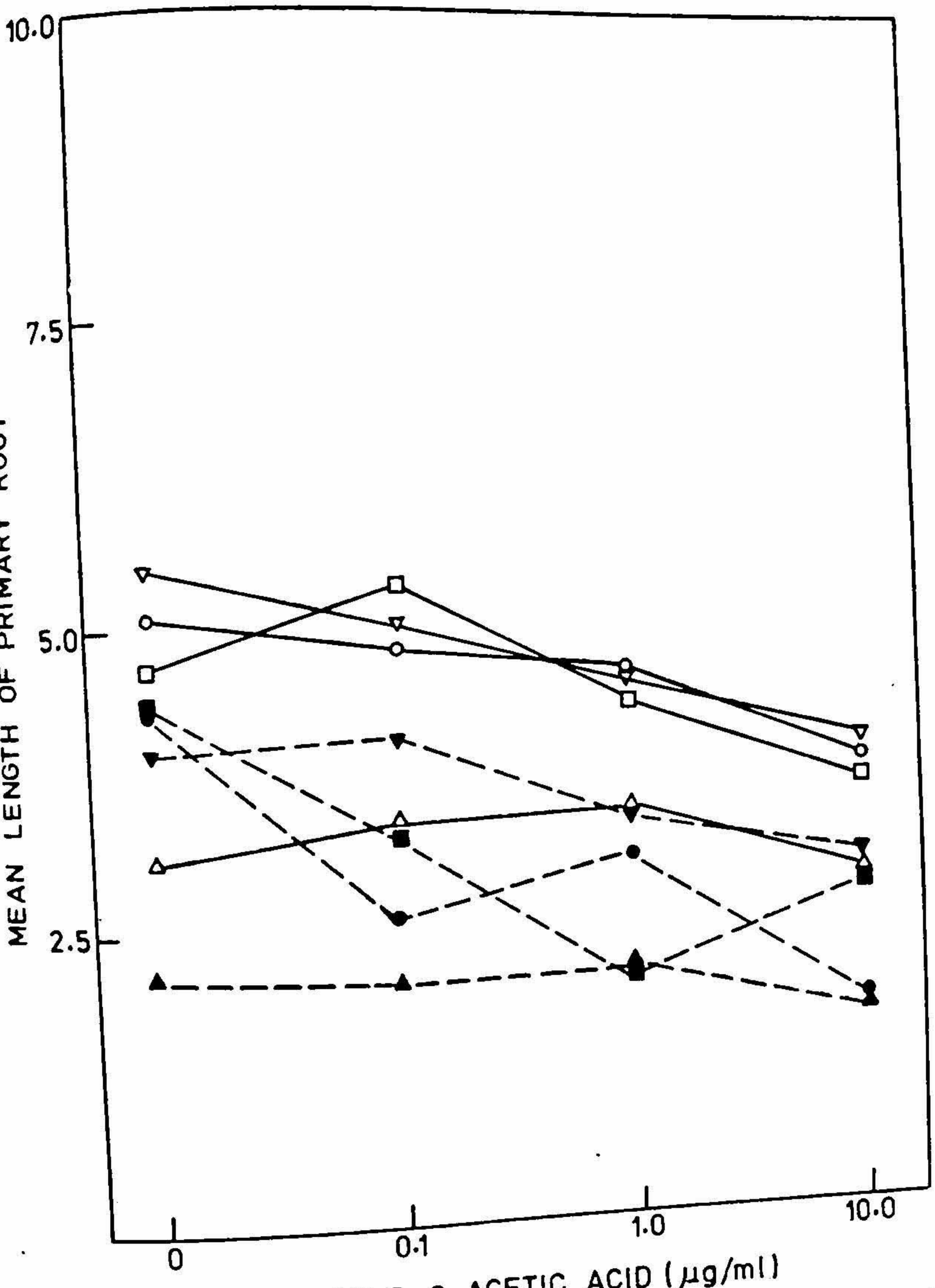

FIG. 6. Effect of light, fensulfothion and indole -3- acetic acid on the development of primary root in pea. (Straight lines with open figures—without fensulfothion; broken lines with closed figures—with fensulfothion; O—dark; Δ—white; □—red; ▽—far-red. Least significant difference = 1·79 cm.)

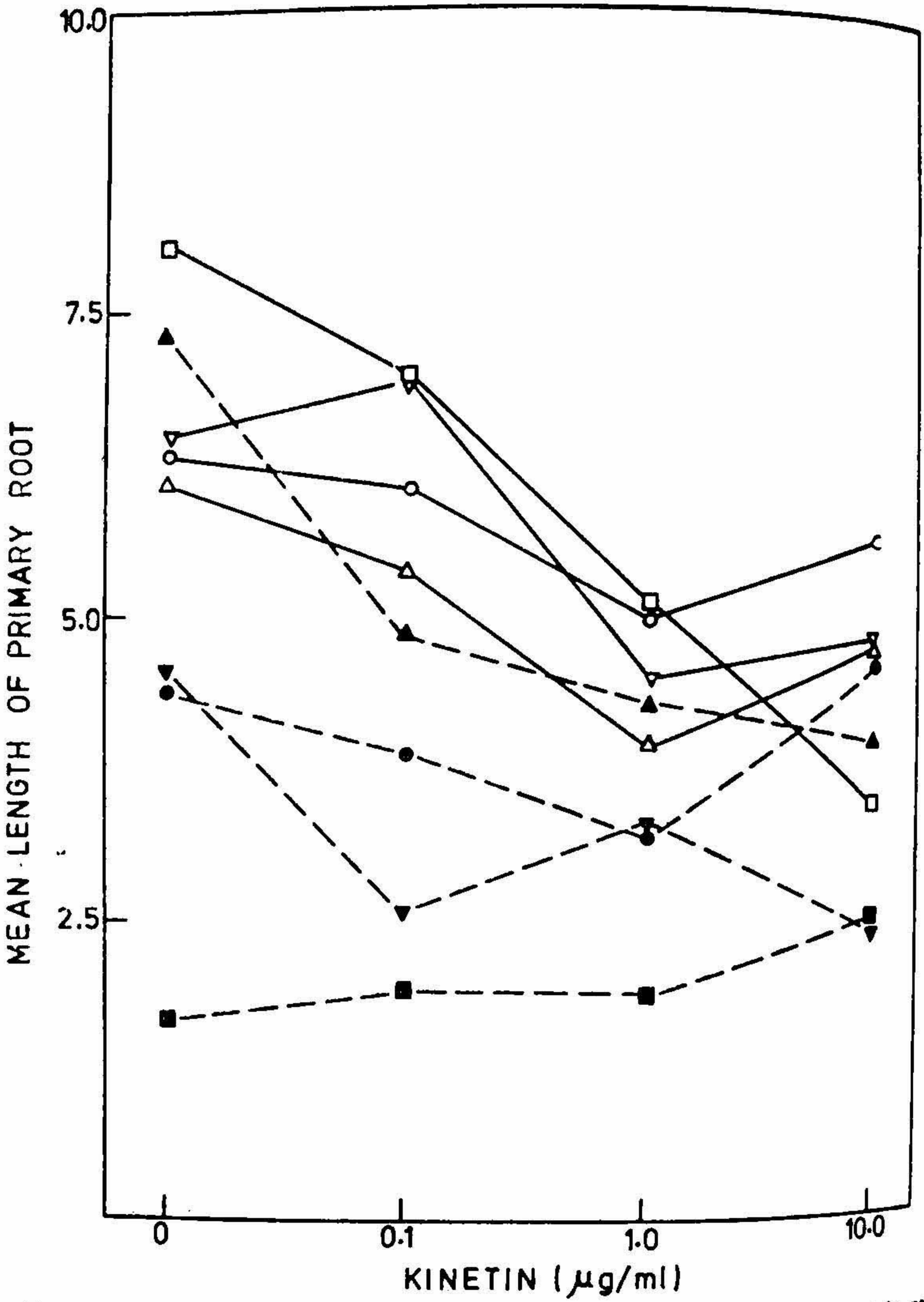

FIG. 7. Effect of light, fensulfothion and kinetin on the development of primary root in pea. (Straight lines with open figures—without fensulfothion; broken lines with closed figures—with fensulfothion; O—dark; Δ—white; □—red; ∇—far-red. Least significant difference = 2·85 cm.)

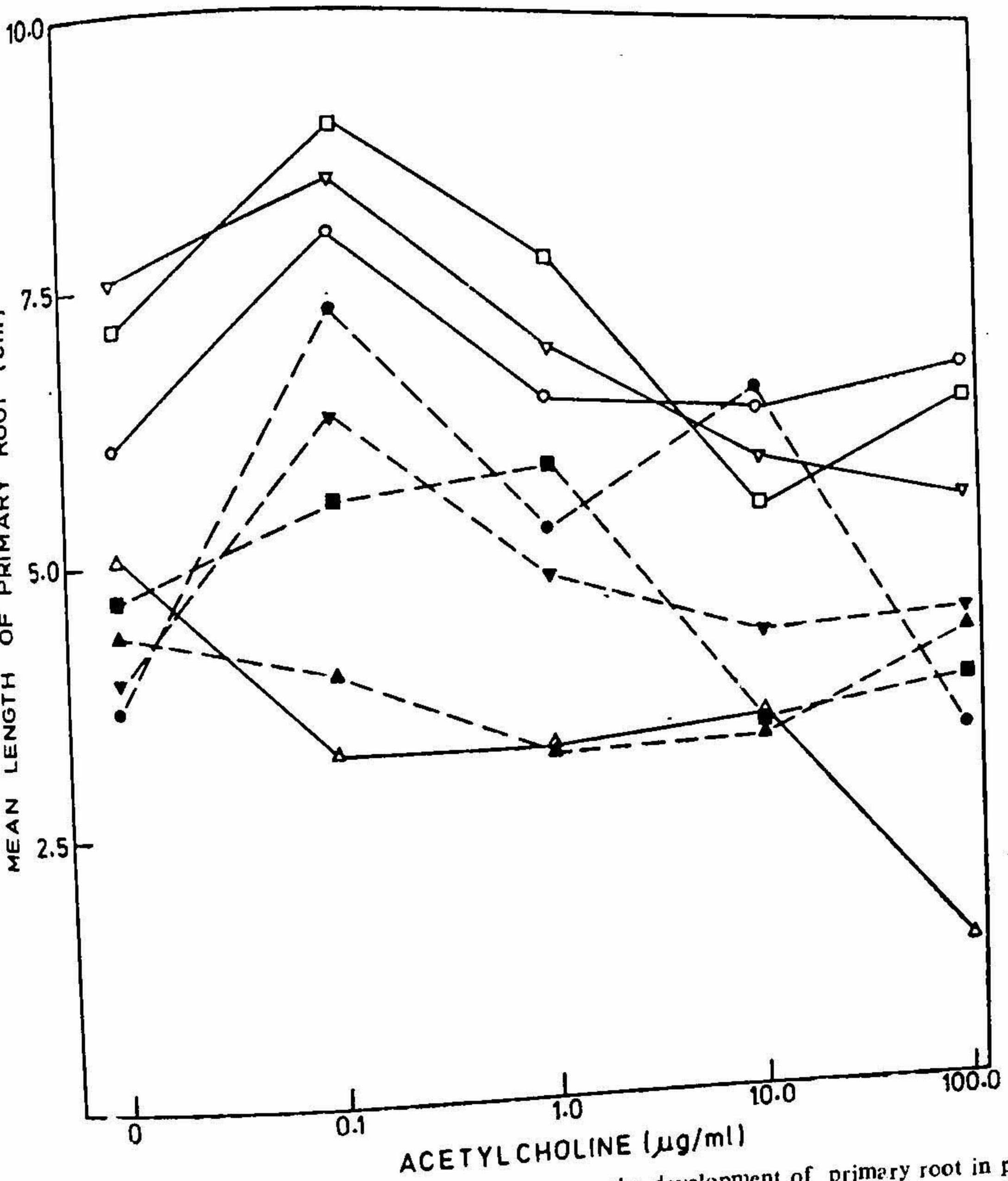

FIG. 8. Effect of light, fensulfothion and acetylcholine on the development of primary root in pea. (Straight lines with open figures—without fensulfothion; broken lines with closed figures—with fensulfothion; O—dark; Δ—white; □—red; ∇—far-red. Least significant difference = 2·17 cm.)

initiated between the dark grown, R or FR light exposed seedlings. However, in the case of white light exposed seedlings the number of secondary roots formed were less in contrast to those exposed to total darkness or R or FR light, as revealed in Figs. 9, 10, 11 and 12.

Effect of light and hormones on secondary root formation : Gibberellic acid, IAA or kinetin treatment did not seem to facilitate better secondary root formation. Increasing

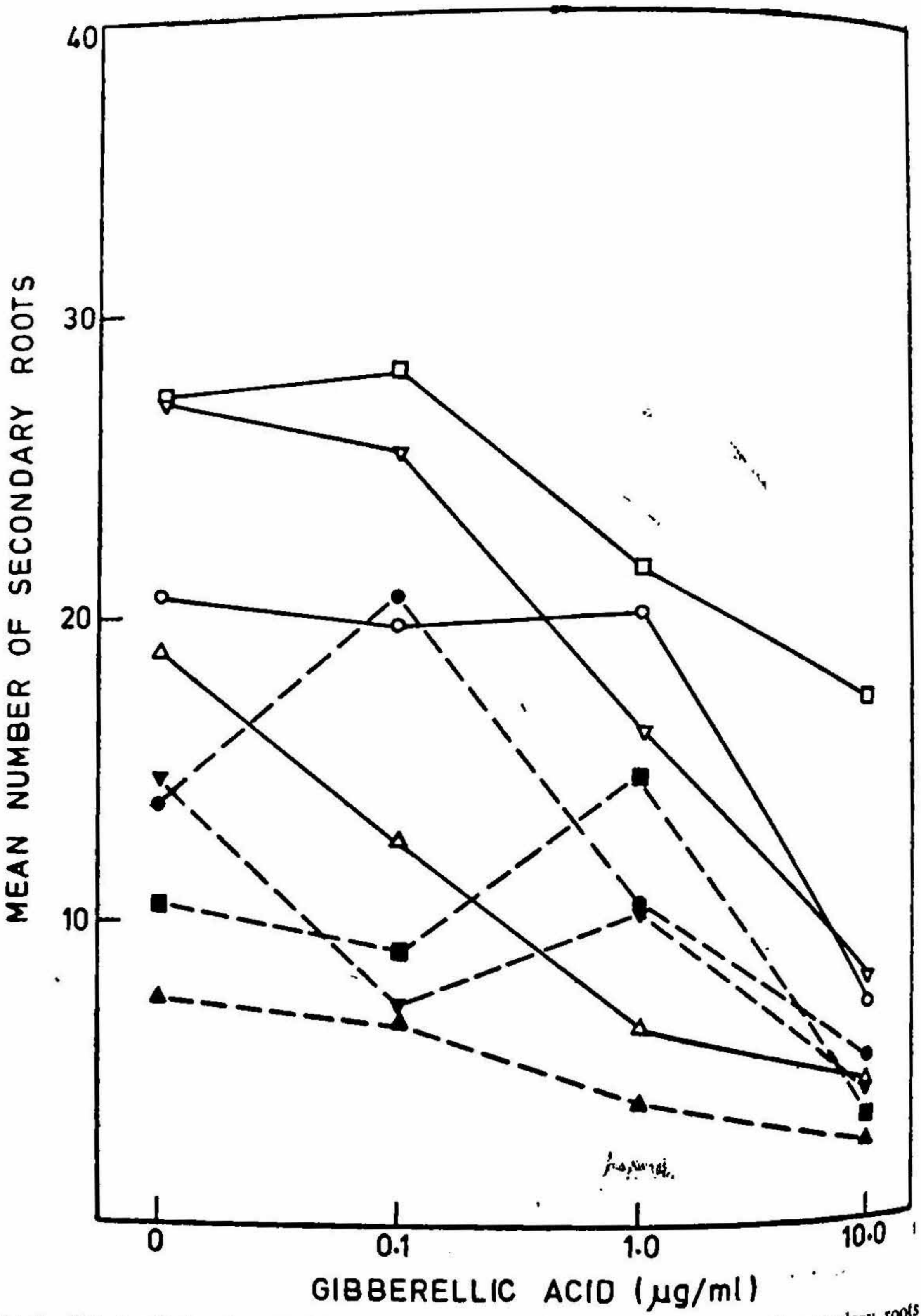

FIG. 9. Effect of light, fensulfothion and gibberellic acid on the development of secondary roots in pea. (Straight lines with open figures—without fensulfothion ; broken lines with closed figures—with fensulfothion ; ○—dark ; △—white ; □—red ; ▽—far-red. Least significant difference = 9·6 cm.)

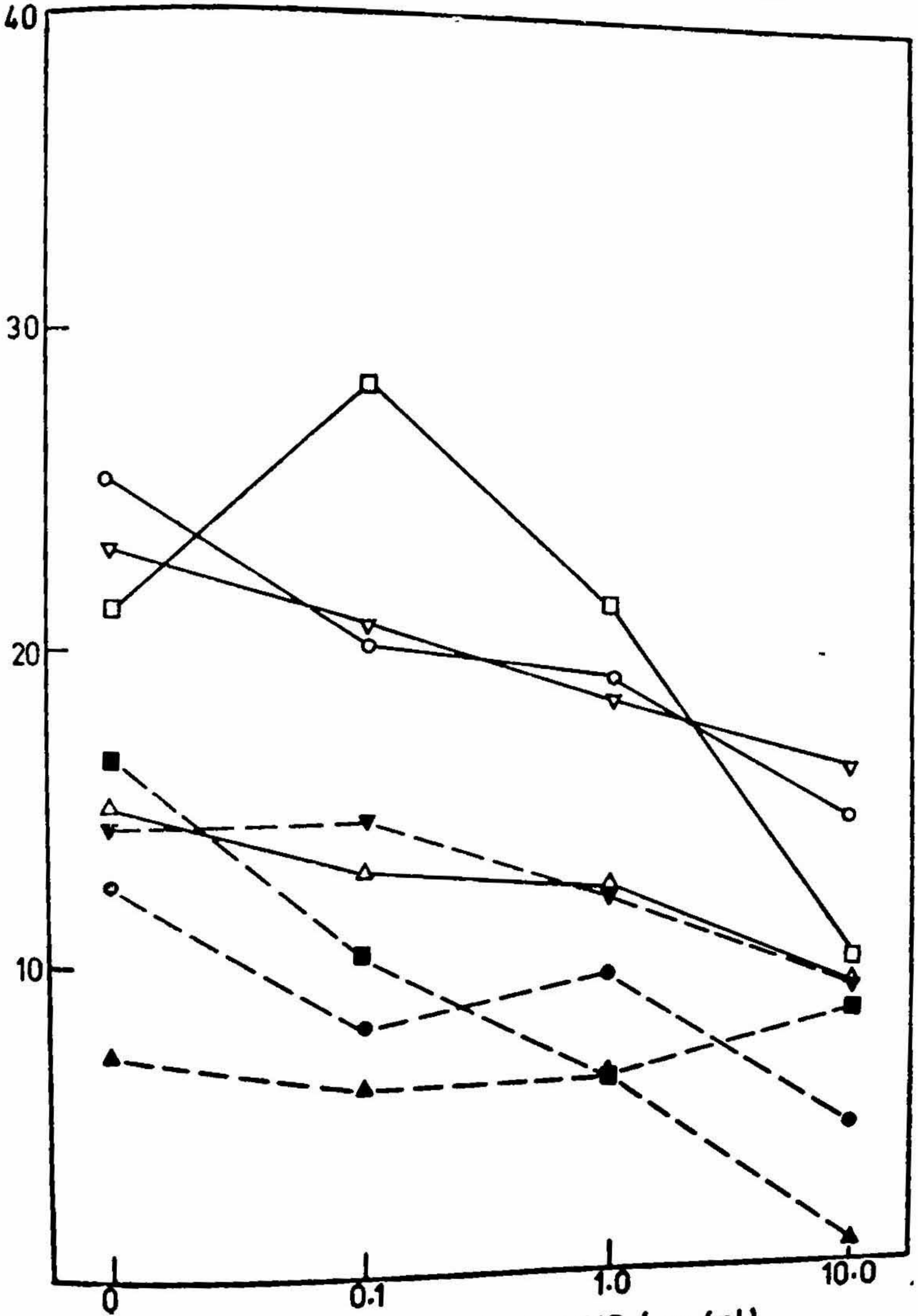

FIG. 10. Effect of light, fensulfothion and indole -3- acetic acid on the development of secondary roots in pea. (Straight lines with open figures—without fensulfothion; broken lines with closed figures—with fensulfothion; O—dark; Δ—white; □—red; ∇—far-red. Least significant difference = 10·7 cm.)

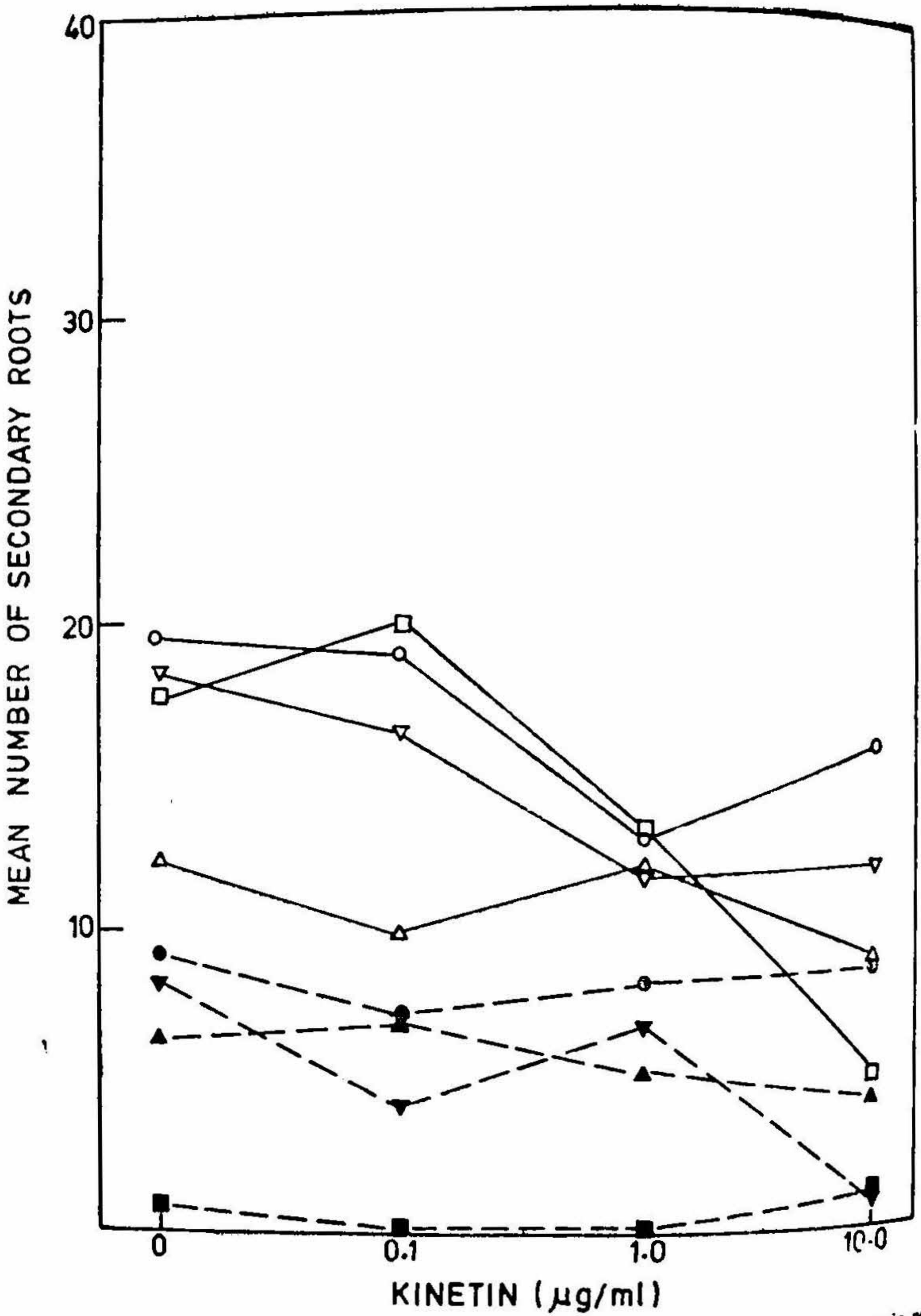

FIG. 11. Effect of light, fensulfothion and kinetin on the development of secondary roots in pea. (Straight lines with open figures—without fensulfothion; broken lines with closed figures—with fensulfothion; O—dark; Δ—white; □—red; ∇—far-red. Least significant difference = 9·08 cm.)

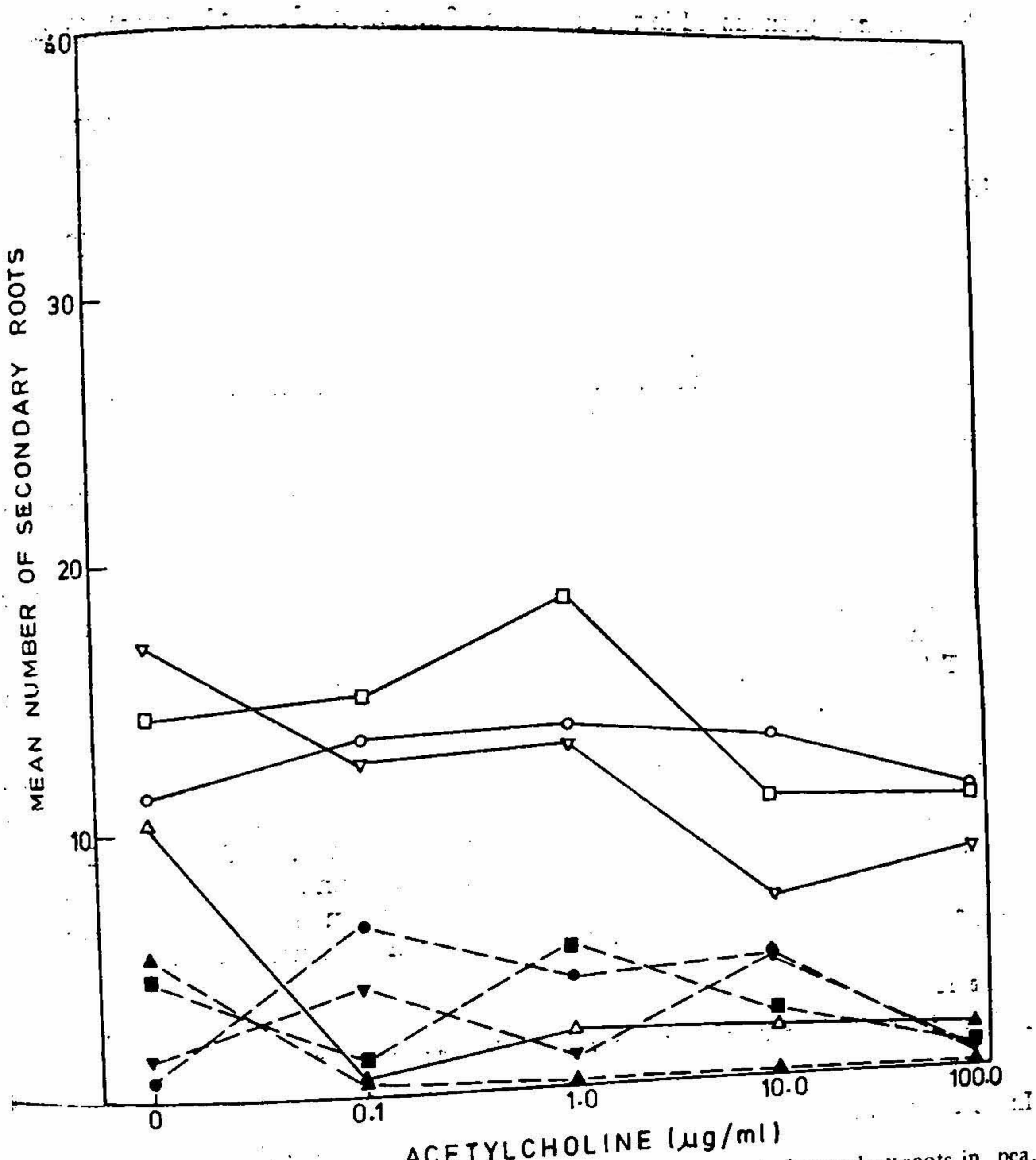

FIG. 12. Effect of light, fensulfothion and acetylcholine on the development of secondary roots in pea. (Straight lines with open figures—without fensulfothion ; broken lines with closed figures—with fensulfothion ; O—dark ; Δ—white ; □—red ; ∇—far-red. Least significant difference = 6·1 cm.)

concentrations of these hormones resulted in the suppression of the number of secondary roots formed (Figs. 9, 10 and 11). Acetylcholine treatment retarded the secondary root formation in white and FR illuminated seedlings (Fig. 12).

Effect of light and fensulfothion treatment on secondary root formation : Fensulfothion treatment drastically reduced the number of secondary roots. The pesticide

effect was most pronounced in the inhibition of initiation of secondary roots (Figs. 9, 10, 11 and 12).

Effect of light, hormones and fensulfothion on the secondary root formation: In the case of fensulfothion treated seedlings, increasing concentrations of GA resulted in the suppression of the number of secondary roots formed under different light conditions. However, at 0·1 μg/ml of GA, in dark grown seedlings, the fensulfothion induced toxicity was overcome (Fig. 9). Indole acetic acid also had the same effect. At high concentration of IAA, the fensulfothion toxicity was enhanced in both dark grown and light exposed seedlings (Fig. 10). It was found that, even kinetin was not able to overcome the fensulfothion induced toxicity at the concentrations tried (Fig. 11). In kinetin treated seedlings, the pesticide induced toxicity was more pronounced in all different light treatments than in dark grown seedlings. In fact, in R illuminated seedlings no secondary roots were formed. The influence of ACh on secondary root formation in fensulfothion treated seedlings was dramatic. It enhanced the fensulfothion toxicity in all the light treatments (Fig. 12).

Thus, it is apparent that during the very early stages of growth soon after germination, 50 μg/ml of fensulfothion retards shoot and root elongation as well as secondary root formation. This toxicity was found to be severe with respect to secondary root formation (Table II). The toxicity due to fensulfothion could not be overcome to significant extent by the addition of various phytohormones and ACh.

Exposing the seeds to different light conditions caused different effects. A summary of the interaction among the three factors *i.e.*, light, hormones and fensulfothion on the growth and development of pea seedlings is presented in Table III. The interaction among light, fensulfothion and hormones was found to be highly significant, especially in ACh treatment, where maximum interaction among ACh, light and fensulfothion on the growth and development of pea seedlings was seen.

Table II

*Effect of fensulfothion on the growth and development of pea**

Light treatment	Shoot	Primary root	Secondary root
Dark	67	72	42
White	75	89	49
Red	63	63	38
Far-red	69	71	42

* The values of no hormone controls from all the experiments were pooled and the results presented are the pesticide treated plants expressed as the peroentage of treated over the untreated plants.

Table III

Abstract of analysis of variance data of the effect of light, hormone and pesticide on the the growth and development of pea

Interaction	Gibberellic acid			Indole acetic acid			Kinetin			Acetylcholine		
	Shoot	Primary root	Secondary root	Shoot	Primary root	Secondary root	Shoot	Primary root	Secondary root	Shoot root	Primary	Secondary root
Light			*	***	*	*	*			***	***	***
Pesticide				*						*		*
Hormone		**	***	**	***	*	*	*	*	*	**	
Light × Pesticide	**		*				*	*	*	*	*	*
Light × Hormone	**	*	*	**	*		**	*		***	***	***
Hormone × Pesticide										*		
Light × Hormone × Pesticide		*		*	*						*	

* Significant at 5% level.

** Significant at 1% level.

*** Significant at 0·5% level.

4. Discussion

It is evident from the results that fensulfothion is toxic to growth and development of pea seedlings. Several explanations have been given for the biochemical basis of inhibition of seed germination and seedling growth and development by organophosphorous pesticides. The pesticides are known to affect a number of processes like, inhibition of a number of hydrolytic enzymes, interfering with the functions of hormones, membrane transport and translocation phenomena and energy metabolism.

The growth and developmental processes are regulated by the complex interactions between various hormones and light[21, 25–27]. In fact, in spite of the extensive studies on the above phenomena, one finds a monotonously repetitive theme, *i.e.*, the diversified functions controlled by the hormones are sometimes overlapping, sometimes opposing and sometimes each hormone controls the functions of the other, either directly or indirectly. These, in turn, are controlled by the amount and the type of light, the plant receives. In this array of phenomena, a general poison, like, an organophosphorous pesticide may affect many of these processes either specifically or non-specifically. It may affect many of the enzymes, notably, acetylcholinesterase[30]. In addition, it might affect several unknown functions and disturb the normal development. Occasionally, such toxicities of pesticides have been alleviated by the addition of various hormones[5]. This could be due to making available the hormone whose synthesis might have been inhibited by the pesticide. Atternatively the addition of hormone externally might be inducing the production of the enzyme(s) which the pesticide might be inhibiting. It is well known that organophosphorous pesticides are anti-acetylcholinesterase compounds which inhibit acetylcholinesterase activity by phosphorylating it. Acetylcholinesterase has been shown to be present in several plants[10–19]. The probable biochemical target for fensulfothion toxicity in pea is also found to be acetylcholinesterase as in animals[18, 24, 31]. However, in this paper only the data concerning the phytotoxic effect of fensulfothion as well as the interactions among light, hormones and fensulfothion in affecting the growth and development of pea are presented. These data often show that there are complex interactions between light and hormones. With the pesticide the situation had become much more complex.

The retardation of shoot growth by fensulfothion is seen both in dark and light exposed seedlings (Table III). The shoot elongation is more under the control of GA than on IAA or cytokinins. The results also show that the recovery is better in GA treatments than in the others. Root elongation is highly sensitive to the presence of the pesticide, often there being complete stunting of growth. Inhibition of root growth has been observed in several pesticide treated plants and this could be due to interference of several hormonal and light functions. It is likely that the effect on the shoot growth is a reflection of shoot growth for not only nutrient uptake during the early stages but even hormone production is monitored by the growing root tip and GA is synthesized in large amounts in the root tip.

The most significant effect of pesticide application was noted with lateral root formation. It is also most profoundly affected by ACh than by any of the other hormones tested. In the absence of the pesticide, ACh had no effect in dark grown seedlings, but increasing concentrations of ACh steadily decreased the number of roots formed in FR and white light treated seedlings. In red illuminated seedlings there was an increase up to 1 μg/ml followed by a reduction under still higher concentrations.

It has been shown that fensulfothion gets metabolized in plants, into its oxygen analogues like, sulfoxide, sulfone and *o*-sulfone and also it is hydrolyzed to *p*-methyl-sulfinyl-phenol and di-ethyl phosphorothioic acid[32, 33]. Most of these metabolites, particularly sulfoxide and *o*-sulfone, are highly toxic to animals. However, the influence of these metabolites on plants is not known. But several phenols and phenolic substances are known to affect the synthesis and the activity of phytohormones which regulate the activity of many enzymes. At the moment, it is not clear whether fensulfothion itself or its metabolized products like, sulfoxide, sulfone, *o*-sulfone and *p*-methyl-sulfinyl-phenol are involved in the phytotoxicity, though acetylcholinesterase has been shown to be one of the major targets.

5. Acknowledgement

The author is thankful to Dr. V. N. Vasantharajan, Microbiology and Cell Biology Laboratory, Indian Institute of Science, Bangalore, for encouraging her to initiate this problem, and for valuable suggestions and continued guidance. The author is thankful to the University Grants Commission for financial assistance.

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