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Integrated Pest Management Strategy (IPM) for Control of *M. scalaris* Attacking Oyster Mushroom (*Pleurotus Ostreatus*) in Western Kenya

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Abstract:

M. scalaris is a pest of economic importance in oyster production in western Kenya but adapted pest control measures have been associated with individual shortcomings. The aim of the current study is to develop an integrated pest management (IPM) strategy that will provide efficient pest control measure against *M. scalaris* while favoring growth and yield of oyster mushroom. Oyster mushroom (*Pleurotus ostreatus*) was established at three sites across three seasons in this area in a Randomized Complete Block Design. Pest control measures:- three layered nylon netting material (physical barrier), Decis (synthetic insecticide), potted Mexican marigold (*Tagetes minuta*) + High spawn rate (8% w/w spawn), IPM [three layered nylon netting material + potted Mexican marigold (*Tagetes minuta*) + High spawn rate (8% w/w spawn)], High spawn rate (8% w/w spawn) and Normal spawn rate (6% w/w) (check) were treatments replicated three times. *M. scalaris* were sampled, identified and counted weekly per treatment. Days to: - full spawn run, pin head formation, first and second flushes as well as fresh weight and %BE was recorded. Data collected was analyzed using GENSTAT statistical software version 12 and significant differences tested at 5% level of significance. Mean reactions were separated by contrast comparison. Incidences of *M. scalaris* across treatments showed significant differences ($p < 0.05$). IPM and Decis (insecticide) had lowest *M. scalaris* incidences compared to Normal spawn rate but IPM had highest fresh weight (131.4gms) and %BE (26.3) with shortest duration to various developmental stages of oyster mushroom. A mean separation of these two-treatment comparison in terms of incidences of *M. scalaris* was not significantly different ($p < 0.05$) while days to developmental stages and yield of oyster mushroom between these two treatments was significantly different ($p < 0.05$). Thus, IPM managed *M. scalaris* while enhancing growth and yield of oyster mushroom.

Keywords: IPM, *M. scalaris*, *Pleurotus ostreatus*

1. Introduction

M. scalaris has been identified as a pest of economic on production of oyster mushroom in western Kenya (Juma *et al.*, 2015). Both the flies and the larvae of *M. scalaris* cause direct and indirect damage on oyster mushroom. The *M. scalaris* larva feeds on the oyster mushroom mycelium resulting to a reduction of the amount mycelium inoculum colonizing the substrate. This equally reduces the vigor of mycelial growth leading to slow colonization of the substrate thus slow spawn run lengthening the duration to full spawn run and consequently the duration of the subsequent developmental stages of the oyster mushrooms. Apart from the direct damage associated with *M. scalaris*, the indirect damage characterized by the excreta secreted by the *M. scalaris* larvae alters the physical and chemical properties of the substrate making the substrate unfit for colonization by the oyster mushroom mycelium. This is in addition to the fact that *M. scalaris* flies are key vectors of *Pygmephorus* spp. which is equally a pest of economic importance in oyster production in this region (Juma *et al.*, 2015). The incidence of *M. scalaris* in western is affected by the seasons in this area and the age of the mushroom farm. As a result, there is need to develop an efficient pest control measure against *M. scalaris* with reference to the seasons in western Kenya and age mushroom farms. This will enhance the production of oyster mushroom while reducing the damage associated with *M. scalaris* on oyster mushroom production in western Kenya. The pest control measures that have been adapted by farmers in this region had been associated with individual short comings in control this pest where

some of these pest control measures have being report to negatively affect growth and yield of oyster mushroom despite controlling the pests (Ogotu perscomm). The aim of this study is to enhance the control of *M. scalaris* using an integrated pest management (IPM) strategy as IPM will combine pest control measures that are compatible, safe, sustainable and ecologically sound to enhance control of *M. scalaris*. Equally the integration of these control measures in an IPM strategy will allow the integrated pest control measures to complement each other's individual shortcomings while providing an efficient pest control measure that will favour the growth and yield of the mushroom crop while discouraging the infestation and prevalence of *M. scalaris*.

2. Materials and Methods

The study was conducted in Butere Constituency in Kakamega County under the Marama West Mushroom Growers Group farmers. Plastic bag technology was used in this experiment where *Pleurotus ostreatus* was established at three sites across the three seasons in western Kenya in Randomized Complete Block Design between September 2012 and August 2013. Pest control measures;- three layered nylon netting material (physical barrier), Decis (synthetic insecticide), potted Mexican marigold (*Tagetes minuta*) + High spawn rate (8% w/w spawn), IPM [three layered nylon netting material + potted Mexican marigold (*Tagetes minuta*) + High spawn rate (8% w/w spawn)], High spawn rate (8% w/w spawn) were the treatments replicated three times. All the treatments were compared to 6% w/w spawn rate as a control, a rate that is used by farmers in the area (Ogotu perscomm). Spawn rate was used as a pest control measure since it is a cultural pest control measure in mushroom production. Sugarcane leaves were used as substrate and were sourced locally chopped, soaked and drained overnight. Wheat bran 100 g and 10 g of lime was added to 1kg wet weight of the substrate after which the substrate was packed into clear polythene bags of 1kg net wet weight and the necks tied using polythene strands. The substrate was pasteurized with steam and left to cool for 24hrs. Spawning of the substrate was done based on the treatments and the bags were labeled. In the decis treatment, decis was incorporated into the substrate after cooling at a dosage of 0.4ml/1litre (Babar *et al.*, 2012) where 10mm was applied to 1kg wet weight of the substrate before being spawned. Potted Mexican marigold was introduced into the production units based on the treatments. The bags were incubated in the dark at $25\pm 2^{\circ}\text{C}$ to allow for mycelial colonization of the substrate. The end of the incubation period was marked by dense white mycelium covering the entire substrate bag. The growing rooms were kept humid at 85-90% Relative Humidity with a 12h light/12h dark photoperiod at $23\pm 2^{\circ}\text{C}$. The growing rooms were watered twice-thrice daily to enhance the humidity and induce fruit body formation. The mushrooms were harvested for two flushes and the substrate blocks were destroyed.

Each treatment was inspected weekly for *M. scalaris* infestation where infested substrate bags per treatment were isolated and *M. scalaris* adult flies were extracted under sterile conditions using an aspirator. The sampled *M. scalaris* were sorted identified and counted where the numbers were recorded on weekly basis per treatment across the seasons.

Days to full spawn run, days to pin head formation after spawn run, days to the first and second flushes was recorded to evaluate effect of the treatments on growth of oyster mushroom. The fresh weight and biological efficiency (%BE) were calculated using the formulae outlined by Palapala *et al.*, (2006) to assess the yield of the mushroom across the treatments.

Data collected was analyzed using GENSTAT statistical software version 12 and significant differences were tested at 5% level of significance while mean reactions were separated by contrast comparison. Data on *M. scalaris* that were sampled was subjected to a logarithmic transformation [$\text{Log}_{10}(x+2)$] before being subjected to analysis of variance (ANOVA).

3. Results

3.1. Incidences of *M. Scalaris* across the Pest Control Measures

The incidences of *M. scalaris* across the pest control measures (treatments) showed significant differences ($p < 0.05$). The incidences of *M. scalaris* within the treatments across the seasons per site showed almost the same trend where IPM and decis (insecticide) had the lowest incidences of *M. scalaris* followed by Mexican marigold + High spawn rate, High spawn rate and Nylon netting respectively compared to Normal spawn rate (Figure 1). Although IPM and decis had lower *M. scalaris* incidences across the seasons per site, a mean separation of these two-treatment comparisons was not significantly different ($p < 0.05$) in terms of incidences of *M. scalaris*.

But the incidences of *M. scalaris* in a mean separation of comparisons between IPM and Mexican marigold + High spawn rate; High spawn rate and Nylon net; High spawn rate and Normal spawn rate; Normal spawn rate and decis; Mexican marigold + High spawn rate and Nylon net showed significant differences ($p < 0.05$). This was similar to a mean separation of comparisons between IPM and High spawn rate; IPM and Nylon net; IPM and Normal spawn rate; IPM and Mexican marigold + High spawn rate which showed significant differences ($p < 0.05$) in terms of the incidences of *M. scalaris*.

Figure 1: Mean Number of Incidences of *M. scalaris* within Pest Control Measures (Treatments) across the Three Seasons per Site, A- Old Mushroom Farm, B- On-Station and C- New Mushroom Farm

3.2. Growth and Yield of Oyster Mushroom across the Pest Control Measures

The duration to the various developmental stages of oyster mushrooms based on days to full spawn run, pinning, first flush and second flush across the treatments showed significant difference ($p < 0.05$) (Table 1).

Oyster mushrooms took the longest duration to various developmental stages under Nylon netting compared to Normal spawn rate (Table 1) followed by High spawn rate and Decis treatment respectively. Oyster mushrooms under *M. marigold* + High spawn rate treatment took shorter time to the various oyster mushroom developmental stages compared to Normal spawn rate but slightly longer time compared to IPM compared to Normal spawn rate.

A mean separation of days to full spawn run, pinning, first flush and second flush in comparisons between Decis and High spawn rate; Normal spawn rate and Nylon netting; IPM and *M. marigold* + High spawn rate was not significantly different ($p < 0.05$). But a mean separation of days to full spawn run, pinning, first flush and second in a comparison between IPM and Decis showed significant difference ($p > 0.05$) while comparisons between Nylon net and Decis; IPM and High spawn rate; IPM and Normal spawn rate; IPM and Nylon net showed significant difference ($p < 0.05$).

TREATMENTS	DAYS TO			
	FULL SPAWN RUN	PINNING	FIRST FLUSH	SECOND FLUSH
High spawn rate	33	36	40	45
Normal spawn rate	43	47	52	59
Decis (insecticide)	32	35	39	45
IPM	26	28	31	36
<i>M. marigold</i> + High spawn rate	29	32	35	40
Nylon net	40	43	48	54
MEAN DAYS	33	36	40	45
S.E	0.197	0.150	0.196	0.190
S.E.D	0.279	0.211	0.277	0.269
F.Probability (TREATMENTS)	<0.001	<0.001	<0.001	<0.001
%CV	3	3	3.5	3

Table 1: Mean Table of Days to the Various Developmental Stages of Oyster Mushroom per Treatment

In terms of yield of oyster mushroom, the fresh weight (gms) and %BE across the treatments showed significance difference ($p < 0.05$) (Figure, 3) respectively. Oyster mushrooms under decis (insecticide) treatment had the lowest fresh weight (gms) and a %BE in comparison to Normal spawn rate followed by Nylon net, High spawn rate and Mexican marigold + High spawn respectively. Oyster mushrooms in IPM treatment had the highest fresh weight (gms) and a %BE in comparison to Normal spawn rate (Table 2).

A mean separation of fresh weight (gms) and %BE in comparisons between IPM and M. marigold +High spawn rate; Normal spawn rate and Nylon net was not significantly different ($p < 0.05$) while comparisons between Nylon net and Decis; IPM and High spawn rate showed significant difference ($p > 0.05$). But a mean separation of fresh weight (gms) and %BE in comparisons between Decis and High spawn rate; M. marigold+ High spawn rate and Normal spawn rate; IPM and Decis; IPM and Normal spawn rate; IPM and Nylon net showed significant difference ($p < 0.05$).

TREATMENTS	FRESH WEIGHT (GMS)	BIOLOGICAL EFFICIENCY (BE%)
High spawn rate	112.5	22.5
Normal spawn rate	83.9	16.8
Decis (insecticide)	86.3	17.3
IPM	131.4	26.3
M. marigold + High spawn rate	117.7	23.5
Nylon net	98.0	19.6
MEAN	103.6	20.7
	FRESH WEIGHT (GMS)	BIOLOGICAL EFFICIENCY (BE%)
S.E	0.133	0.027
S.E.D	0.188	0.038
F.Probability (TREATMENTS)	<0.001	<0.001
%CV	0.9	0.9

Table 2: Mean Table of Fresh Weight (Gms) and BE% of Oyster Mushroom per Treatment

4. Discussion

4.1. Incidences of *M. scalaris* across the Pest Control per Site across the Three Seasons

Nylon netting was characterized by high *M. scalaris* infestation despite the fact that netting at entry points to production units has been used as a physical barrier against flies attacking mushrooms (Annon, 2009). These high incidences of *M. scalaris* were as a result of early *M. scalaris* infestation during cooling of pasteurized substrate and spawning where once the larvae were hatched, they fed on the mycelium despite of the nylon netting as the physical barrier. Also *M. scalaris* successfully penetrated through the 3 layered Nylon netting materials that were used as a physical barrier. This is because *M. scalaris* have been reported to have an extraordinary capacity to even penetrate into and escape eight layers of stretched gauze (Disney, 2008). Equally a double layer of organdy cloth has been found not to control the infestation of *M. scalaris* (Koch *et al.*, 2013). *M. scalaris* might have also infested the mushrooms by penetrating through the necks of the polythene bags used in production of oyster mushrooms since Disney, (2008) reported that *M. scalaris* can invade plastic bags of turtle eggs by crawling through the knots tied at the neck of these bags.

Equally the 6% w/w spawn rate used with nylon netting might have also contributed to the high *M. scalaris* infestation because this spawn rate might have provided low inoculation points of the spawn in the substrate resulting in slow substrate colonization rate (Royse, 2003; Upadhyay, 2006). This resulted to presence of substrate uncolonised by the mycelium that allowed the larvae to successfully feed on the mycelium with minimum mortality. *Megaselia* spp. larvae feed successfully in substrate that is being colonized by mycelium and not already colonized substrate (Royse, 2003). Also, this spawn rate might have not provided sufficient nutrition for dense growth of mycelium in the substrate (Upadhyay, 2006) hence providing optimum condition for the *M. scalaris* larvae to feed successfully. This because the grains used in the spawn are the major source of nutrition for the mycelium during spawn run hence the higher the spawn rate the more the grains thus sufficient nutrition for dense mycelium growth which hampers the feeding of *M. scalaris* larvae.

The 8% w/w spawn rate in the High spawn rate treatment provided more inoculation points of spawn in the substrate enhancing increased colonization of the substrate by the mycelium (Royse, 2003) reducing the amount of uncolonised substrate susceptible to infestation by *M. scalaris* larvae. The 8% w/w spawn rate also provided sufficient nutrition for dense growth of the mycelium in the substrate (Upadhyay, 2006) reducing the susceptibility of such substrate to *M. scalaris* larvae infestation because *M. scalaris* do not feed on densely colonized substrate (Royse, 2003). But lack of nylon netting as a physical barrier and the repellant pungent scent from potted Mexican marigold against *M. scalaris* fly infestation in this treatment hampered its efficiency in controlling *M. scalaris*.

Mexican marigold (*Tagetes minuta*) contains borneol as one of its active ingredients in its pungent scent that has been found to have repellant properties against insect flies (Dipterans) (Wyss, 2011; Taylor, 2013). Hence having *M. scalaris* as a Dipteran, the repellant properties of potted Mexican marigold reduced the infestation of *M. scalaris* flies in the treatment that entailed High spawn rate+ potted Mexican marigold.

Decis (deltamethrin) significantly reduced the infestation of *M. scalaris* by inhibiting the female *M. scalaris* from laying eggs on the substrate since female *Megaselia* spp. do not lay eggs on chemically treated substrate (Babar *et al.*, 2012).

The integration of Nylon netting, potted Mexican marigold and High spawn rate in the IPM treatment significantly controlled *M. scalaris* as this treatment recorded low incidences of *M. scalaris* flies. The 8%w/w spawn rate provided more spawn inoculation points in the substrate for rapid colonization of the substrate as well as providing sufficient nutrition for dense growth of the mycelium thus reducing the susceptibility of the mushroom to *M. scalaris* larvae infestation (Keil, 2002). The fumes from the potted Mexican marigold served as a repellent against the *M. scalaris* flies while nylon netting although not conclusively provided some physical barrier against the few *M. scalaris* flies that sneaked into the production units. Thus, these integrations of Nylon netting + potted Mexican marigold + High spawn rate in IPM provided an all-round management of *M. scalaris* across the seasons per site.

4.2. Growth and Yield of Oyster Mushroom across the Pest Control Measures

Nylon netting was characterized by a lengthened duration to the various developmental stages of oyster mushrooms. This was due to the high incidences of *M. scalaris* in this treatment. The *M. scalaris* larvae fed on the oyster mushroom mycelium resulting to a reduction of the amount mycelium inoculum colonizing the substrate. This equally reduced the vigor of mycelial growth leading to slow colonization of the substrate thus slow spawn run lengthening the duration to full spawn run and consequently the duration of the subsequent developmental stages of the oyster mushrooms (Babar *et al.*, 2012). Apart from the direct damage associated with *M. scalaris*, the indirect damage characterized by the excreta secreted by the *M. scalaris* larvae altered the physical and chemical properties of the substrate making the substrate unfit for colonization by the oyster mushroom mycelium (Babar *et al.*, 2012). Thus, the vigor of mycelium growth through these contaminated portions of the substrate reduced lengthening the duration spawn run during incubation and consequently lengthening the duration to the subsequent developmental stages of oyster mushrooms. These reduced vigor and density of the mycelia growth equally led to fruiting bodies of low fresh weight (gms) and %BE. The 6% w/w spawn rate in these treatments also contributed to this low fresh weight (gms) and %BE by equally lowering the vigor and the density of mycelium growth due to the low nutrients provided by the less grains used in these 6% w/w spawn rate.

Decis (insecticide) was characterized by low incidences of *M. scalaris* but a lengthened duration to the various developmental stages of oyster mushrooms. This treatment might have altered the chemical and physical properties of the substrate causing inhibited mycelial growth hence delayed fruiting of the oyster mushrooms (Shamshad, 2010). As a result, the duration of oyster mushroom to full spawn run, pin head formation, first and second flush lengthened that consequently impacted on the yield leading to low the fresh weight and the %BE of the harvested mushrooms.

The role of 8% w/w spawn rate in High spawn rate and High spawn rate + Potted Mexican marigold treatments to enhance the vigor and density of the mycelium growth for shortened duration to the various developmental stages of oyster mushrooms, high fresh weight (gms) and high BE% was not fully realized. This might be due to the damage associated by the few incidences of *M. scalaris* in this treatment in that their damage is still significant even at their low infestation levels since the economic threshold of these two pests is still unclear (Annon, 2013; Annon, 2009). This led to slightly lengthened duration to the several developmental stages of oyster mushroom in these treatments with low fresh weight and % BE as compared to IPM treatment. Although this is in spite of the fact that as the spawn rate increases the duration of *Pleurotus* spp. to full spawn run and subsequent developmental stages shortens (Chauhan, 2013). Thus, in this study the damage associated with even one *M. scalaris* might be of economic importance in oyster mushrooms. The shortened duration to the various developmental stages of oyster and high fresh weight (gms) and %BE of IPM was not only due to the almost zero incidences of *M. scalaris* in this treatment but was also due to the 8% w/w spawn rate in this treatment.

The 8%w/w spawn rate enhanced the vigor and density of the mycelium growth by providing sufficient nutrients to the mycelium due to the more grains in these 8% w/w spawn rate hence resulting to shortened duration to the various developmental stages with fruiting bodies of high fresh weight (gms) and %BE (Upadhyay, 2006). This is in addition to the fact that the damage associated with *M. scalaris* on oyster mushroom in this treatment was also reduced. Thus, IPM enhanced the growth of oyster mushrooms by shortening the growth duration hence not only enhancing quick returns on investment but also reducing build up of pest population associated by delayed spawn run and fruiting (Musieba *et al.*, 2012; Royse, 2003).

5. Conclusion

The integration of Nylon netting + potted Mexican marigold + High spawn rate in IPM provided an all-round management of *M. scalaris* across the seasons per site while enhancing growth and yield of oyster mushroom.

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